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## BIOLOGICAL DECOMPOSITION OF CHEMICAL LIGNIN

### I. SULPHITE WASTE LIQUOR[1]


BY G. A. ADAMS[2] AND G. A. LEDINGHAM[3]



### Abstract

The wood staining fungus *Endoconidiophora adiposa* was found to be capable of decomposing approximately 10% of the lignin fraction of sulphite waste liquor media as measured by the β-naphthylamine precipitation method. It was also capable of utilizing 10 to 15% more of the reducing sugars than yeast. In the preparation of a suitable culture medium a number of different neutralizing agents were tested but, provided the proper pH adjustment was attained, there was little difference in their effect. Phosphate and nitrogen compounds were the only supplementary nutrients required and, for the fungus tested, organic and ammonia nitrogen was more satisfactory than inorganic nitrates.


### Introduction

Sulphite waste liquors have long been regarded as an important industrial waste product and a great deal of research has been done to find a profitable and successful method of utilizing, or even disposing of, the enormous volumes of this material. For every ton of sulphite pulp manufactured, approximately 10 tons of sulphite liquor are produced, containing on an average 10 to 12% solids, of which 60% is the lignin fraction and 15 to 20% the soluble sugars.

In Canada at present there are two plants making baker's yeast from the sugars present in the liquor and one plant producing chemicals from the lignin fraction, but these industries use a relatively small amount of the available waste liquor. It must be recognized that the utilization of the sugar still leaves the lignin fraction to be disposed of as waste. In recent years considerable research has been carried out in an attempt to find uses for this calcium lignosulphonate as a chemical product. One of the most successful developments has been the Howard process (5) for making lignosulphonic acids.

Few attempts have ever been made to determine whether or not the lignosulphonates can be broken down by the action of micro-organisms. Since they are water soluble, these compounds are more adaptable to fermentation experiments than most forms of chemical lignin. Benson (2) demonstrated that the organic matter content of Puget Sound never increased in


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spite of the large amounts of sulphite waste liquor being dumped into the water, and concluded that naturally occurring organisms were decomposing it. On the basis of this observation he made a study of the problem, using mixed cultures of anaerobic bacteria from sea mud, and found that the amount of gases formed could not be accounted for on the basis of fermentation of the sugars alone (3). So far as the action of fungi on lignosulphonate is concerned, the only measurements made appear to be those of Kazanskii and Mikhailova (7). They tested only a few species of wood destroying fungi. More work has been done on the decomposition of acid, alkali, and phenol lignin (10, 12), and, in general, chemical lignin has been found to be very resistant to decomposition by micro-organisms. Pringsheim and Fuchs (11) found that alkali lignin on being incubated with forest soil was rendered partially alcohol soluble. Norman (8) states that the consensus of opinion is that normal isolated lignin is unavailable to micro-organisms. There has, of course, been a great deal of work done on the breakdown of the various plant constituents in composts and soils and on such economic problems as the rotting of wood. The results, so far as lignin is concerned, are by no means clear-cut. Some workers claim rapid disappearance of lignin and others find little or no change.

Since little work has been done on the decomposition of the lignin fraction of sulphite waste liquor, it appeared that a study of this problem might provide further fundamental information on the process of lignin decomposition and the products formed. Few of the factors that affect the progress of the decomposition have been investigated thoroughly, hence it would appear that if suitable media for growth of fungi or bacteria could be made from the lignin fractions of sulphite waste liquor, some of these fundamental questions could be investigated and a search made for the most active lignin destroying micro-organisms. The first object of this investigation was to determine whether microbial decomposition of lignosulphonates was feasible and to make quantitative estimations of the process. Much preliminary work was necessary for the selection of suitable organisms and the preparation of a satisfactory medium for their growth from sulphite waste liquor.

## Preparation of Culture Media

Before sulphite waste liquor can be used as a culture medium for the growth of micro-organisms, it is necessary to remove the toxic substances such as the free sulphur dioxide, adjust the pH, and supply certain essential nutritional elements which are lacking.

As measured by the iodine method (1) the free sulphur dioxide content of the raw sulphite liquor used in these experiments was 0.122%. Boiling with vigorous aeration for 15 min. reduced this figure to 0.039%. The same treatment for four hours gave 0.028%. Using the oxidizing agents potassium permanganate, hydrogen peroxide, and sulphuric acid, the values of 0.034, 0.036, and 0.026%, respectively, were obtained. Concentration of the raw liquor under reduced pressure was effective in reducing the sulphur dioxide

content, but neither it nor the methods in which oxidizing agents were used were sufficiently superior to the simple 15-min. boiling and aeration treatment to warrant their continued use.

The pH of sulphite waste liquor after removal of the free sulphur dioxide by 15 min. of boiling is 2.80 to 2.90. In order to have satisfactory media for the growth of most fungi, this must be raised to approximately 5.0. This can be accomplished by the addition of bases, such as calcium, barium, magnesium, sodium; and ammonium carbonates and hydroxides, sodium bicarbonate, and calcined brucite. Sulphite liquor media neutralized with these substances alone, or in combination, were tested for suitability in so far as growth of fungi was concerned. Brucite, calcium, magnesium, sodium, and ammonium carbonates were selected for subsequent experiments in which quantitative determinations of pH change and of sugar and lignin losses were made.

Sulphite waste liquor is deficient in nitrogen and phosphorus, both of which are essential for the growth of fungi. It is therefore necessary to add optimum amounts of these in a form in which they are readily available. The following nitrogen compounds were tested: ammonium sulphate, nitrate, carbonate, monohydrogen phosphate, dihydrogen phosphate, potassium nitrate, casein, peptone, several amino acids, and urea. The phosphorus was usually added in the form of phosphate, although very good results may be obtained with phosphoric acid. The sources used were sodium and potassium mono- and dihydrogen phosphates, phosphoric acid, and magnesium glycero-phosphate. All of these were satisfactory, but the ammonium phosphates had the advantage of supplying nitrogen in the same compound and keeping the salt content of the media at the lowest level. Mono- and dihydrogen phosphates alone or in combination were used in order to obtain desired pH levels.

## Methods and Analytical Procedure

In most of the experiments the raw liquor was boiled for 15 min. with vigorous aeration to remove sulphur dioxide. The neutralizing agents were added to the hot solution and the mixture allowed to stand overnight. The supernatant liquor was poured off and filtered through "Supercel" on a Buchner funnel. If the pH was not satisfactory, further neutralizing material was added and the mixture refiltered. One hundred ml. amounts of this solution were pipetted into 500 ml. Erlenmeyer flasks and sterilized for 20 min. at 120° C. The necessary nutrients, which had been separately sterilized, were added after cooling.

The organism used in these experiments was *Endoconidiophora adiposa* (Butler) Davidson, a wood staining fungus which grew rapidly and produced a smooth surface mat. Culture flasks received an inoculum of 1 ml. of a spore suspension of the fungus and the controls 1 ml. of sterile water.

The flasks were incubated in two large water baths in which the temperature was accurately controlled to within $\pm 0.1°$ C. and the humidity of the

air was high enough to prevent undue evaporation during the growth period. Difficulty was experienced with contaminating organisms growing down through the cotton plug of the flask. This was overcome by igniting the cotton plug as soon as the cultures were sterilized and immediately capping it with a waxed paper drinking cup.

Triplicate pairs of control and inoculated flasks were randomized as to position in the bath. The analysis included measurements of pH, reducing sugars, and calcium lignosulphonate.

The pH was determined by means of a Beckmann pH meter using a glass electrode. Reducing sugars were determined by the method outlined by Partansky and Benson (9).

A survey of the available methods for the analysis of calcium lignosulphonate indicated that the method devised by Hägglund *et al.* (4) and later modified by Partansky and Benson (9) was most suitable. Some changes were introduced. The essential difference between the procedure used and that outlined by Partansky and Benson was the use of 15 ml. of β-naphthylamine for the precipitation, as it was found that 10 ml. of reagent was not sufficient to produce as complete precipitation of the calcium lignosulphonate as was possible with the larger amount. Additional amounts did not produce more precipitation. The second difference was the shortening of the air drying of the precipitate from 12 to 24 hr. to one hour, and the using of the oven drying period of three to four hours. Any loss in volume due to evaporation during the incubation period was taken into account in the final calculation.

The results obtained by using the modified procedure were uniform and consistent enough to warrant its use for the determination of relative lignin content of the samples used in these experiments. Fundamentally, it cannot be considered a method that yields absolute values because the precipitate of β-naphthylamine lignosulphonate is not of constant composition and the factors by which it is converted into terms of lignin are open to criticism.

## Experimental

### 1. Effect of Growth Period and Incubation Temperature on Lignin Decomposition

The first experiment was designed on data already accumulated from qualitative experiments in which satisfactory growth of the fungus had been obtained. Several different fungi were tested and *Endoconidiophora adiposa* was chosen because it gave rapid growth and produced a firm felty mat on the surface of the medium and could be readily separated from the medium before analyses were made. The length of growth period, and the effect of two different temperatures, were the factors investigated in the first quantitative experiments. The liquor was treated with 5 gm. per litre of calcium carbonate and magnesium carbonate, respectively, to neutralize the acids. The pH was 7.29. After autoclaving, 5 ml. of a 2.4% solution

of ammonium monohydrogen phosphate was added to each flask. Eighteen inoculated and 18 control flasks were placed in each of the two water baths held at 25° and 30° C. Analyses were made at the beginning of the experiment, and at 5, 10, 20, 35, and 70 day intervals for determination of pH change and sugar and lignin losses. The results are shown in Table I.

TABLE I

THE EFFECT OF TEMPERATURE AND LENGTH OF GROWTH PERIOD ON THE DECOMPOSITION OF SULPHITE WASTE LIQUOR BY *Endoconidiophora adiposa*

| Growth period, days | Temperature, 25° C. | | | | Temperature, 30° C. | | | |
|---|---|---|---|---|---|---|---|---|
| | pH | | Sugar fermented, % | Lignin decomposed, % | pH | | Sugar fermented, % | Lignin decomposed, % |
| | Controls | Test | | | Controls | Test | | |
| 0 | 6.21 | 6.21 | 0.00 | 0.00 | 6.09 | 6.09 | 0.00 | 0.00 |
| 5 | 6.27 | 6.01 | 14.51 | 0.23 | 5.95 | 5.80 | 14.07 | 0.00 |
| 10 | 6.10 | 6.29 | 46.98 | 1.42 | 5.74 | 6.26 | 53.17 | 0.96 |
| 20 | 4.49 | 6.40 | 82.03 | 1.73 | 4.60 | 6.53 | 76.71 | 4.90 |
| 35 | 5.12 | 6.90 | 84.93 | 8.11 | 5.10 | 7.37 | 82.33 | 5.39 |
| 70 | 5.47 | 7.50 | 82.39 | 7.79 | — | 7.35 | 86.61 | 9.31 |

There was a small initial difference in the pH values between the flasks at 25° and 30° C. The pH values showed some irregularities, but the general trend was downward in the controls and upward in the inoculated flasks. The downward trend in the controls was probably due to the slow oxidation of the sulphur compounds to sulphuric acid. The initial drop in pH in the cultures was due to the accumulation of carbon dioxide and organic acids produced in the fermentation. After 10 days this stage was over and the pH increased.

Almost 50% of the fermentable sugars are removed in 10 days and 80% in 20 days. Since yeast is able to utilize only 60% of the total sugars, it is apparent that *E. adiposa* is more efficient in this respect.

The lignin in the control flasks did not remain constant but underwent a steady apparent increase up to about 20 days and then became stabilized. The order of the increase was 3 to 4% of the weight of calcium lignosulphonate in solution. This increase was calculated as the difference between the lignin content of the uninoculated control at the beginning of the experiment and at any other time during the course of the experiment. Since the loss in lignin could not be determined as the difference between the amount in solution at the beginning of the experiment and the amount present in the inoculated solutions at any time during the experiment, it was estimated as the difference between an inoculated and control solution as found at any time during the growth period. The lignin loss in 35 days at 25° C. was about 8%. At 70 days there was no further breakdown of the lignin, which indicated that the greater part of the decomposition occurred prior to 35 days and then gradually ceased. The final loss in lignin was about two to three times

the magnitude of the apparent increase. This indicates that the loss is not a reversal of the apparent increase after a growth period of several weeks, but represents an actual decomposition of the lignin. Between 25° and 30° C. there was no significant difference in so far as lignin decomposition is concerned.

### 2. The Effect of Different Neutralizing Agents

*Effect of Three Different Neutralizing Agents at Two Temperatures and the Same pH*

Brucite, ammonium carbonate, and a mixture of calcium carbonate and magnesium carbonate were used as neutralizing agents. Each experiment was carried out at two different temperatures, 25° and 30° C., and each series was analysed at three time intervals, initial time, 10 days, and 20 days. The pH of the different media were adjusted to approximately the same level.

The addition of 5 gm. per litre of calcium carbonate and 8 gm. per litre of magnesium carbonate to the first series and 20.5 gm. freshly calcined brucite to the second series gave pH values of 6.50 and 5.98, respectively. After sterilization and addition of 5 ml. of a sterilized mixture of 1.2% each of ammonium mono- and dihydrogen phosphate to each 100 ml. of the medium the pH values were 5.50 and 5.67. In the third series the ammonium carbonate was sterilized separately and added with the phosphates at the rate of 1.2 gm. per litre. This gave a final pH of 5.71. The results of the analyses are summarized in Table II.

As has been observed in previous experiments, after incubation the control flasks tended to become slightly more acid. The test flasks, on the other

TABLE II

THE EFFECT OF pH AND COMPOSITION OF MEDIA ON GROWTH OF *E. adiposa* ON SULPHITE WASTE LIQUOR MEDIA

| Series No. | Growth period, days | Temperature, 25° C. | | | Temperature, 30° C. | | |
|---|---|---|---|---|---|---|---|
| | | pH | Sugar fermented, % | Lignin decomposed, % | pH | Sugar fermented, % | Lignin decomposed, % |
| 1 | 0 | 5.50 | 0.00 | 0.00 | 5.80 | 0.00 | 0.00 |
| | 10 | 5.91 | 62.70 | 2.85 | 5.54 | 49.53 | 2.82 |
| | 20 | 6.88 | 74.24 | 8.08 | 6.77 | 76.96 | 8.68 |
| 2 | 0 | 5.67 | 0.00 | 0.00 | 5.45 | 0.00 | 0.00 |
| | 10 | 5.24 | 64.03 | 0.51 | 5.49 | 47.90 | 2.01 |
| | 20 | 6.62 | 72.52 | 4.17 | 6.46 | 80.82 | 6.50 |
| 3 | 0 | 5.71 | 0.00 | 0.00 | 5.62 | 0.00 | 0.00 |
| | 10 | 5.39 | 84.55 | 8.62 | 4.80 | * | * |
| | 20 | 5.66 | 89.32 | 8.78 | 4.88 | * | * |

**Cultures did not grow.*

hand, became more basic and at 20 days had a pH of approximately 6.90. The more extensive the growth of the fungi, the higher the pH was found to be.

The sugar content at the beginning of the experiment was about 22.0 gm. per litre. This remained fairly constant throughout the course of the experiment in the control flasks. In the inoculated cultures at 10 days over 50% of the sugar had been fermented and at 20 days this had risen to 80%, which appears to be about the limit for *E. adiposa*.

The lignin values at the beginning agree very closely and show a content of about 62 gm. per litre. The lignin content of raw sulphite liquor is very close to this figure, so it can be assumed that the addition of calcium and magnesium carbonate in quantities sufficient to bring the pH up to 5.5 to 6.0 does not cause any precipitation of the lignosulphonate. The loss of lignin is approximately the same at both 25° and 30° C.

In the second series the pH in the control flasks showed a progressive increase in acidity, while the test flasks on the other hand showed a decrease in acidity to an average pH of 6.70. Those with poor growth had a correspondingly lower pH. The rate and extent of lignin decomposition was about 50% less than found in the previous series. While the growth on media neutralized with brucite was quite satisfactory, it was apparent from the variations between the individual controls that this neutralizer was interfering with the lignin determinations. Consequently it was not used for further experimental work.

In the third series growth was very rapid at 25° C. and, as indicated by the amount of sugar fermented, was complete in 10 days. There was relatively little change in pH during the experiment. At 30° C., however, the medium became quite acid (pH 4.80) and no growth occurred.

At 10 days the lignin in the controls had undergone an apparent increase from an initial 62 gm. per litre to 64 gm. per litre. In the inoculated flasks the lignin was still about 6 gm. per litre lower than the corresponding controls. At 20 days there was little further change in the loss of lignin in the test flasks. This seems to indicate that the decomposition of lignin paralleled the fermentation of sugar and when the sugar had been removed the decomposition of lignin almost ceased. In the first series in which the sugar was removed more slowly, the lignin was reduced slowly at first and then more rapidly as the sugar reserve was used up. It may be possible that the organism requires some fermentable sugars as a source of energy while adapting itself to the utilization of lignin. If the sugar is rapidly fermented, it appears to leave the organism unable to continue the lignin fermentation effectively.

A comparison of the different treatments carried out in this experiment shows that the best results have been obtained by using a mixture of calcium and magnesium carbonates as the neutralizing agents. The brucite treatment introduced technical difficulties in the determination of lignin. The ammonium carbonate made the nitrogen content unreliable after sterilization

due to loss of ammonia. The difference in the breakdown of lignin for 25° and 30° C. was not significant enough to warrant further use of the higher temperature. In fact, the organism failed to grow in the third series at the higher temperature.

*The Effect of Basic Carbonate Neutralizers*

In this experiment *E. adiposa* was grown in liquor that had been neutralized with sodium, calcium, or magnesium carbonate. The pH was adjusted to a different level with each material.

The addition of 3.4 gm. per litre anhydrous sodium carbonate, 6.5 gm. per litre calcium carbonate, and 6 gm. per litre magnesium carbonate before sterilization gave final pH values of approximately 5.5, 5.9, and 6.8, respectively. all instances 5 ml. of a sterile solution containing 1.2% each of ammonium mono- and dihydrogen phosphate was added to each 100 ml. of media. In this experiment the flasks were incubated at 25° C. only.

The results obtained are reported in Table III. In control flasks neutralized with sodium carbonate the pH dropped to 5.0 and remained fixed there. With calcium carbonate the pH dropped to 5.3 and became stationary. In the test flasks, after the usual initial drop, the pH rose.

TABLE III

THE EFFECT OF DIFFERENT NEUTRALIZING REAGENTS AND pH ON DECOMPOSITION OF SUGARS AND LIGNIN OF SULPHITE WASTE LIQUOR BY *E. adiposa*

| Neutralizing agent | Growth period, days | pH | Sugar fermented, % | Lignin decomposed, % |
|---|---|---|---|---|
| $Na_2CO_3$ | 0 | 5.52 | 0.00 | 0.00 |
| | 10 | 5.37 | 82.30 | 8.27 |
| | 20 | 5.77 | 88.52 | 10.92 |
| $CaCO_3$ | 0 | 5.90 | 0.00 | 0.00 |
| | 10 | 6.12 | 73.26 | 4.63 |
| | 20 | 7.02 | 87.84 | 8.82 |
| $MgCO_3$ | 0 | 6.84 | 0.00 | 0.00 |
| | 10 | 6.38 | 60.95 | 4.64 |
| | 20 | 7.38 | 83.46 | 6.58 |

The fermentation of sugar was most rapid with sodium carbonate. In media treated with both sodium and calcium carbonate the fermentable portion of their sugar was removed in 20 days. Fermentation was considerably slower in the magnesium carbonate media. It appeared that the high initial pH had a retarding effect on growth.

The greatest lignin decomposition occurred in the media neutralized with sodium carbonate; the least in that neutralized with magnesium carbonate.

From Table III it may be seen that the pH of the sodium carbonate is lower than that of the magnesium carbonate media, and so it is possible to

correlate lignin decomposition and pH of the media in this experiment. It is believed that the final pH of the solution is a more important factor than the reagent used to obtain it. While it would be preferable to study the effect of pH alone by using different amounts of the same neutralizing agent, it was found experimentally that only a very narrow pH range could be obtained since sterilization of the media tended to bring them all to the same level. It was therefore necessary to use different neutralizers for each level of pH. For *E. adiposa* the best pH for sugar fermentation and lignin decomposition was around 5.50 to 5.85 but mycelial growth was most rapid at a pH about 6.50.

### 3. The Effect of Different Nitrogen Sources

The nitrogen source is a very important factor in the growth of *E. adiposa* in waste sulphite liquor. Qualitative experiments previously carried out demonstrated that different sources of nitrogen did not give the same amount of growth on agar media. Ammonium nitrogen was one of the best sources, while inorganic nitrate was one of the poorest. A quantitative experiment was set up using six different nitrogen sources.

The culture medium was prepared as previously and neutralized with 5 gm. per litre each of calcium carbonate and of magnesium carbonate. The phosphate source was added before sterilizing as a mixture of 1.2% potassium monohydrogen and 1.2% of dihydrogen phosphate at the rate of 5 cc. of this mixture to each 100 cc. of the medium. The nitrogen sources used were ammonium monohydrogen phosphate, sulphate, carbonate, potassium nitrate, Difco bacto-peptone, and urea. They were added in such quantities that the nitrogen content of the medium would be 4 gm. per litre. In order to keep the phosphate content equivalent in all media, none was added to that containing the ammonium monohydrogen phosphate. The nitrogen solutions were sterilized by filtration through a Jena sintered glass filter. The analyses for pH change, sugar, and lignin content were done at 10 and 20 days. In each set there were two test flasks and one control. The pH of all cultures was approximately 6.00. All were incubated at 25° C. The results obtained are shown in Table IV.

The flasks containing potassium nitrate as a source of nitrogen showed very little growth and the mat was submerged. In all others, growth was fairly rapid, and in about three days the surface of the medium was covered with mats of about equal depth. At the end of 10 days the pH value had undergone little change. At 20 days there was a marked change in pH, most of the cultures having become alkaline with the exception of those containing potassium nitrate and ammonium monohydrogen phosphate. At 20 days, the sugars, except in the potassium nitrate culture, were about 80% utilized; this, as previously observed, is about the maximum fermentation of the sugars of waste sulphite liquor by *E. adiposa*. In the potassium nitrate media, on which growth was poor, about 50% of the utilizable sugar was still left. The general appearance of the other cultures indicated that

TABLE IV

THE EFFECT OF NITROGEN SOURCES ON THE DECOMPOSITION OF SUGARS AND LIGNIN OF SULPHITE WASTE LIQUOR BY *E. adiposa*

| Nitrogen source | Initial pH | Analysis at 10 days | | | Analysis at 20 days | | |
|---|---|---|---|---|---|---|---|
| | | pH | Sugar fermented, % | Lignin decomposed, % | pH | Sugar fermented, % | Lignin decomposed, % |
| $(NH_4)_2HPO_4$ | 6.10 | 5.89 | 36.92 | 3.76 | 5.86 | 79.89 | 0.00 |
| $(NH_4)_2SO_4$ | 6.00 | 6.40 | 55.86 | 5.23 | 7.45 | 83.75 | 9.41 |
| $(NH_4)_2CO_3$ | 6.38 | 6.56 | 59.62 | 6.00 | 9.11 | 80.93 | 7.07 |
| $KNO_3$ | 6.00 | 5.66 | 13.42 | 1.36 | 5.67 | 40.11 | 2.66 |
| Peptone | 6.25 | 6.50 | 56.28 | 5.45 | 7.48 | 86.10 | 8.13 |
| Urea | 6.10 | 6.49 | 48.42 | 4.53 | 7.45 | 82.19 | 9.07 |

the active growth period was over as they had turned from white to dark mats which were wrinkled at the edges. The lignin breakdown at 20 days was somewhat greater than at 10 days with the exception of the ammonium monohydrogen phosphate flasks, which after showing a breakdown of 3.76% lignin in 10 days, at 20 days showed no lignin breakdown at all. This appears to be due to a change in metabolism as the pH of this solution never became alkaline as in the others. The precipitate that was formed with β-naphthylamine was not the typical plastic-like substance usually obtained, but was a powdery, reddish material, which weighed more than its control, thus giving the illusionary effect of lignin having been built up again at 20 days. In view of the fact that in the experiments with different neutralizers ammonium monohydrogen phosphate, when used in larger quantities gave excellent growth, rapid sugar fermentation, and lignin breakdown of 8 to 10%, the poor results here may be attributed to the smaller amount used in this experiment. It must be pointed out that this ammonium monohydrogen phosphate medium is not strictly comparable to the others since in the other cultures phosphates were present in the form of potassium salts. The lignin breakdown in the potassium nitrate cultures was negligible. Of the other sources of nitrogen there does not appear to be any preference, all being utilizable and to approximately the same extent. The ammonium sulphate gave the best breakdown, with urea next. Peptone also appears to be a good source of nitrogen.

Less extensive experiments carried out with the amino acid glycine and the amine, asparagine, proved that they are also available sources of nitrogen for this organism.

## Discussion

Sulphite waste liquor may be readily converted into a suitable culture medium for the growth of fungi. Since the lignin is present in the form of water soluble calcium lignosulphonate, it is more adaptable for studies on decomposition than insoluble lignin compounds. One of the main disadvantages in using sulphite waste liquor is the fact that the lignin content, as measured by the β-naphthylamine precipitation method, undergoes an

apparent increase over a period of approximately three weeks in sterile culture media. This fact might lead to the conclusion that the loss of lignin may be only a reversal of the apparent increase and that no real loss had occurred. However, it has been shown that the calculated loss is from two to three times the amount of the increase and thus indicates that an actual loss of calcium lignosulphonate has taken place. Further evidence for a real decomposition of the lignin is provided by the relation between growth and lignin breakdown. When growth of the fungus is abundant as with suitable sources of nitrogen, the lignin loss is correspondingly greater than with poor nitrogen sources which give scanty growth. The fact that extensive growth is positively correlated with lignin decomposition indicates that the lignin is being utilized in the process of growth.

It will be observed that the lignin breakdown in most cases parallels the sugar fermentation and after the available sugar has been used up, the lignin decomposition tapers off rather quickly. It was thought that it might be possible to stimulate the breakdown of lignin if more sugar were supplied, qualitative tests having shown that available nitrogen and phosphate still remained. Sugar was added in amounts of 1, 3, and 5% to cultures that had been fermenting slowly for six months. Analysis showed they consumed the sugar completely, but there was no further breakdown in the lignin. This would lead to the supposition that only a certain fraction of the lignosulphonate is fermentable and when that part has been consumed the reaction stops. It may be that this conversion yields compounds like humic acid that are very resistant to microbial growth. The nature of the precipitate obtained with $\beta$-naphthylamine on these old cultures lends strength to the idea that the lignosulphonate has been changed considerably as it does not give a hard brittle plastic-like precipitate but a powdery grit-like substance. There is evidence to show that in native lignin decomposition it combines with proteins to give substances that are very resistant to bacterial action (6, 13). It is possible that the same type of reaction may occur when fungi are grown on waste sulphite liquor.

It is important to point out that consistent results in the experiments with different nitrogen sources have been very difficult to obtain. Attempts were made to have the actual amounts of nitrogen in the media identical and this has meant that other factors, probably of equal importance, have been thrown out of their proper proportion. For other reasons yet unexplained, the results have sometimes been poor, for instance, the lignin breakdown with ammonium monohydrogen phosphate in the experiment on nitrogen sources was nil although in previous experiments it has been possible to get a breakdown of 6.7%. Sulphite liquor has such a variety of different substances in its composition that it is difficult to make an identical medium for each experiment and so little is known of the metabolism of *E. adiposa* that slight changes may profoundly influence its growth. Only a further study of the problem will elucidate all the factors governing the growth of the organism in this medium.

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# BIOLOGICAL DECOMPOSITION OF CHEMICAL LIGNIN

## II. STUDIES ON THE DECOMPOSITION OF CALCIUM LIGNOSULPHONATE BY WOOD DESTROYING AND SOIL FUNGI[1]

By G. A. Ledingham[2] and G. A. Adams[3]


## Abstract

The growth and lignin decomposing properties of 106 cultures of wood destroying and soil fungi have been studied on a synthetic calcium lignosulphonate medium. Certain species of *Fusarium* and *Alternaria*, decomposing a maximum of 12 and 18% lignin, respectively, were the most effective in utilizing the lignosulphonate. Although a few species of wood destroyers were equally effective, in general this group of fungi showed great variation and was more difficult to cultivate on the medium employed. A slight positive correlation was found between the Bavendamm tannic acid reaction for identifying lignin decomposing fungi and the lignosulphonate breakdown after 60 days' growth.


In a previous paper (1) it was demonstrated that the wood staining fungus *Endoconidiophora adiposa* (Butler) Davidson was capable of decomposing approximately 10% of the calcium lignosulphonate of sulphite waste liquor, provided the liquor had been rendered non-toxic and was supplemented with certain essential nutrients. However, it was found that the complex character of sulphite waste liquor presented several problems. The lignosulphonate content of the control media did not remain constant but steadily increased over a period of about 30 days. This made dependable appraisal of the results difficult, as the increase was not always constant over several identical controls. Furthermore, the uniform removal of sulphur dioxide from waste liquor to give a non-toxic medium for growth of many different organisms could not always be accomplished satisfactorily. It was thought that these difficulties could be largely eliminated by the use of a medium containing pure lignosulphonate salts isolated from sulphite waste liquor. After preliminary studies had been carried out in order to develop a satisfactory standard medium, a survey was made of different species or strains of fungi in order to determine their lignin decomposing properties.

## Methods

### *Preparation of Calcium Lignosulphonate*

Two litres of sulphite waste liquor were evaporated on a hot plate to a volume of 600 ml. and vigorously aerated at the same time. The solution was filtered when cool, and 12 ml. of concentrated sulphuric acid was added with stirring. A heavy white precipitate of calcium sulphate came down, was filtered off, and washed with water. The pH of the filtrate was adjusted to

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approximately 5.00 with calcium carbonate (20 gm.). This pH adjustment was necessary as the strongly acid solution rendered the lignosulphonate soluble in the subsequent treatment with alcohol. The solution was then centrifuged to remove the calcium sulphate. Two hundred and fifty ml. of the solution was then added to two litres of 95% ethanol at the rate of about two drops per second, the alcohol solution being mechanically stirred at a rapid rate at the same time. The calcium lignosulphonate precipitated out in the alcohol solution, but care had to be taken to stop the addition of the liquor before the precipitate became brown and sticky. After pouring the alcohol off, the precipitate was repeatedly washed with 95% ethanol, then taken up in 125 ml. of water and again precipitated from two litres of 95% ethanol. This second precipitate was washed with fresh 95% ethanol and separated on a Buchner filter. The precipitate was then dried in a warm draught of air. When dried and ground, it was a light buff coloured powder, which was stable when stored out of contact with air. The yield was about 60 gm. from two litres of liquor.

*The Isolation and Selection of Lignin Decomposing Fungi*

Fungi are generally regarded as being among the most active of the micro-organisms that break down the native lignin in plant materials in soils and composts. Waksman and Hutchings (7) found that species of *Fusarium* and *Alternaria* could cause decomposition of phenol lignin and Sadasivan (5) has shown that *Fusarium culmorum* (W. G. Smith) Sacc. is an important primary colonizer of wheat straw buried in soil. Cultures for this study were isolated by inoculating sulphite waste liquor media with bits of rotting wood, leaf mould, heating manure, well rotted manure, mouldy hay, straw and cereal grains, rotting fruit and vegetables, and soil. Certain species survived and flourished in this medium and pure cultures of these were isolated. In addition, cultures of wood destroying fungi were obtained from the Division of Botany, Central Experimental Farm, Ottawa, and the Forest Products Laboratory, Washington, D.C. Several species of *Fusarium* were obtained from the Dominion Laboratory of Plant Pathology at Saskatoon, Sask., and strains of *Alternaria*, *Phoma*, *Melanospora*, and a few other genera, many of which undoubtedly occur in soil, were obtained from a collection isolated from seeds in the Division of Botany, Central Experimental Farm, Ottawa.

Forest mycologists have attempted to classify wood destroying fungi into cellulose and lignin decomposing types by growing them on media containing tannic acid. According to Bavendamm (2) who devised the test, those species that produce a brown discoloration on this medium elaborate enzymes that are capable of breaking down lignin. Davidson, Campbell, and Blaisdell (3) tested the reactions of 210 wood destroying fungi on gallic and tannic acid media and found that 80% of the negative reactors were correctly diagnosed as brown rot fungi and 96% of the positive reactors were diagnosed as white rot fungi. Since white rot fungi attack the lignin more actively than the cellulose, it appeared that this test might be useful in selecting lignin decomposing fungi for this work. It also appeared worth while to determine

whether there was any correlation between the reaction on tannic acid media and actual breakdown of lignosulphonate acids.

*Preparation of the Culture Media*

The same medium was used for the growth of all the species tested. Obviously a standard medium could not be expected to be entirely satisfactory for all the different fungi tested as their nutritional requirements are not identical. However, in order to test a large number of species, it was not practical to attempt nutritional studies with each to determine the optimum conditions for growth. The standard medium that was used for this general survey was, however, very satisfactory except for a few species. It was made up as follows:

| | |
|---|---|
| Ammonium monohydrogen phosphate | 5.0 gm. |
| Potassium chloride | 1.0 gm. |
| Magnesium sulphate | 1.0 gm. |
| Ferrous sulphate | 0.02 gm. |
| Dextrose | 20.0 gm. |
| Calcium lignosulphonate | 50.0 gm. |
| Distilled water | 1000 ml. |

The calcium lignosulphonate was added in approximately the same amount as is present in sulphite waste liquor. It should be noted that while 5 gm. per litre of ammonium monohydrogen phosphate would be high in an ordinary medium, there is a heavy precipitate of phosphate produced when this is added to calcium lignosulphonate, hence the amount in solution at any one time is considerably less than 0.5%. It was determined experimentally that this amount gave better growth with most species than a smaller quantity and no detrimental effects were noted even when 10 gm. per litre was added. This culture medium also contains glucose. While it would have been preferable to have had lignosulphonate as the only carbon source, previous studies indicated that the growth without additional carbon was so meagre that little change occurred in the medium. With a readily available carbon source present growth of the fungus was luxuriant, and with those species capable of attacking the lignosulphonate there was a slow but gradual breakdown so long as growth of the fungus occurred.

In preparing the medium, the calcium lignosulphonate and magnesium sulphate were dissolved in half the volume of boiling water and the ammonium monohydrogen phosphate, potassium chloride, and ferrous sulphate in the remainder. Fifty ml. of each solution was then pipetted into a 500-ml. flask. This assured an even distribution of the precipitate that occurred when ammonium monohydrogen phosphate, magnesium sulphate, and calcium lignosulphonate interacted. The medium was then sterilized at 120° C. for 20 min. During sterilization the pH dropped from approximately 7.00 to 5.50.

After cooling, the flasks to be inoculated received 1 ml. of a spore suspension of the organisms being tested. Uninoculated control cultures were incubated with the inoculated. Usually two cultures, with a control, were grown and

analysed, one at each of two dates. However, in a few tests designed to test the accuracy of the method, triplicate cultures of certain species were analysed. Analyses were made at intervals of 10 and 20 days for most of the cultures except the wood destroyers, which, because of slow growth, were done at 30- and 60-day intervals. Whenever an analysis was made, three uninoculated flasks of medium were analysed to give a base line for lignin. The methods of analysis used were the same as those described in the previous paper in this series (1).

## Experimental Results

### The Decomposition of Calcium Lignosulphonate by Soil Fungi

*Species of Alternaria*

Thirty-three isolates of *Alternaria* were tested on the calcium lignosulphonate medium. The analytical results which include changes in the pH of the media and percentage loss of the sugar and lignin at 20- and 30-day intervals are shown in Table I, in decreasing order of lignin loss after 20 days' incubation. The initial pH of the medium was 4.70 and in all cultures the pH had decreased at 10 days. After 20 days' growth it was found that in some instances the pH was higher than its corresponding value at 10 days, while in others it still continued to fall. These pH values are undoubtedly associated with the utilization of the sugar. At 10 days there was a correlation coefficient of $-.578$ between the percentage of sugar fermented and the pH of the solution, which indicates that the most active fermentation produced the greatest acidity. At 20 days the correlation between pH and sugar was .386, indicating that the organisms that had utilized the most sugar had now started utilizing the acids. On the other hand, the percentage of lignin decomposed showed no demonstrable association with either pH or fermentation of sugar at either 10 or 20 days. There was no consistent trend for either pH or lignin, consequently one could not predict at 10 days what the results would be at 20. With sugar there was, however, a significant correlation, though small for purposes of prediction ($r = .51$) between percentage of sugar lost at 10 and 20 days. The 33 cultures of *Alternaria* had marked differences in their ability to decompose lignin, showing losses of 3 to 18% of the lignosulphonate present in the solution.

*Species of Fusarium*

The analytical results obtained from the growth of 13 different species of *Fusarium* on calcium lignosulphonate media are shown in Table II. The medium and experimental conditions were similar to those used with the *Alternaria* species but the growth period was extended to 40 days in order to find out whether a longer fermentation period would influence lignin breakdown.

It is apparent from this table that the sugar fermentation was practically complete at 20 days. In 6 out of the 13 species studied there was an increase in the lignin decomposition at 40 days, as compared with 20 days, but the remainder showed an apparent decrease. This effect was frequently observed

TABLE I

ANALYTICAL RESULTS FROM *Alternaria* CULTURES GROWN ON CALCIUM LIGNOSULPHONATE MEDIA

| N.R.C. culture No. | Incubation time, days | | | | | |
|---|---|---|---|---|---|---|
| | 10 | | | 20 | | |
| | pH | Sugar fermented, % | Lignin decomposed, % | pH | Sugar fermented, % | Lignin decomposed, % |
| *T–G–2* | 4.39 | 42.2 | 8.1 | 4.62 | 98.4 | 18.2 |
| *L–21* | 3.78 | 25.1 | 5.1 | 3.35 | 77.9 | 16.9 |
| *D–96* | 4.05 | 23.7 | 4.1 | 4.01 | 49.3 | 16.8 |
| *T–S–29* | 4.05 | 81.6 | 15.1 | 5.43 | 98.1 | 16.0 |
| *L–119* | 3.60 | 84.5 | 2.4 | 5.50 | 97.6 | 15.3 |
| *L–13* | 3.80 | 32.6 | 7.6 | 3.87 | 46.2 | 15.0 |
| *T–G–36* | 3.55 | 63.4 | 3.7 | 3.85 | 98.4 | 15.0 |
| *P–1* | 3.60 | 21.7 | 6.6 | 3.45 | 89.1 | 14.3 |
| *T–G–26* | 4.59 | 7.8 | 2.6 | 3.70 | 76.8 | 13.6 |
| *T–G–10* | 4.60 | 15.4 | 7.2 | 3.95 | 93.0 | 13.2 |
| *L–52* | 4.02 | 17.0 | 1.4 | 3.59 | 57.6 | 11.8 |
| *T–G–1* | 4.65 | 32.2 | 8.8 | 4.62 | 97.0 | 11.6 |
| *T–G–27* | 4.42 | 21.1 | 5.2 | 4.12 | 95.1 | 11.5 |
| *T–G–23* | 4.58 | 17.1 | 9.7 | 3.72 | 95.2 | 11.1 |
| *L–113* | 3.72 | 45.6 | 3.8 | 3.75 | 98.0 | 10.5 |
| *T–G–29* | 4.28 | 27.4 | 5.1 | 4.00 | 97.5 | 10.3 |
| *L–155* | 3.50 | 71.8 | 1.8 | 3.89 | 97.4 | 10.2 |
| *T–G–38* | 4.18 | 34.7 | 4.8 | 3.75 | 74.9 | 9.9 |
| *T–G–33* | 4.43 | 27.1 | 5.2 | 3.73 | 51.7 | 9.3 |
| *T–G–19* | 4.30 | 28.6 | 4.0 | 5.02 | 97.8 | 9.2 |
| *E–O–2* | 3.72 | 73.6 | 1.2 | 4.35 | 98.7 | 8.7 |
| *T–G–37* | 4.32 | 22.4 | 7.0 | 3.40 | 94.8 | 8.3 |
| *T–G–22* | 4.62 | 25.8 | 7.8 | 3.78 | 95.1 | 8.0 |
| *T–G–35* | 3.50 | 74.5 | 8.2 | 4.60 | 97.6 | 7.7 |
| *L–86* | 3.72 | 12.3 | +1.4 | 3.81 | 38.4 | 7.7 |
| *T–G–3* | 4.60 | 35.2 | 8.2 | 4.22 | 98.4 | 7.5 |
| *T–G–18* | 4.65 | 2.0 | 0.3 | 4.09 | 33.0 | 6.7 |
| *T–G–28* | 4.35 | 29.1 | 4.1 | 4.30 | 97.5 | 6.3 |
| *T–G–34* | 3.75 | 59.2 | 6.0 | 4.10 | 97.6 | 5.1 |
| *T–G–9* | 4.42 | 30.7 | 5.2 | 4.10 | 76.6 | 5.0 |
| *T–G–4* | 4.35 | 63.6 | 3.3 | 4.89 | 98.4 | 4.5 |
| *T–G–25* | 4.35 | 32.5 | 3.3 | 5.05 | 97.4 | 3.0 |
| *T–G–8* | 4.42 | 34.9 | 1.5 | 4.89 | 98.7 | 2.8 |

in cultures that had been growing for a long period of time. The lignin precipitate as obtained by $\beta$-naphthylamine had been changed and became a reddish powder instead of a brittle black plastic. This precipitate weighed more than the corresponding one at 20 days and gave the apparent effect of lignin having been built up during the last 20 days. This may have been due either to products of metabolism precipitating and being calculated as lignin, or to an influence on the precipitation of calcium lignosulphonate so that it was more complete (normally $\beta$-naphthylamine precipitates only 68% of calcium lignosulphonate present).

At 20 days there was a correlation coefficient of .67 between pH and sugar values, and between lignin and pH the value was .53. This means that low pH values were associated with almost complete sugar fermentation

TABLE II

ANALYTICAL RESULTS FROM *Fusarium* CULTURES GROWN ON CALCIUM LIGNOSULPHONATE MEDIA

| N.R.C. culture No. | Species | Incubation time, days | | | | | |
|---|---|---|---|---|---|---|---|
| | | 20 | | | 40 | | |
| | | pH | Sugar fermented, % | Lignin decomposed, % | pH | Sugar fermented, % | Lignin decomposed, % |
| *T–S–24* | *F. culmorum* | 2.92 | 83.9 | 13.4 | 4.25 | 90.0 | 12.5 |
| *T–S–23* | *F. coeruleum* | 3.40 | 90.2 | 8.9 | 3.60 | 93.1 | 11.8 |
| *T–S–17* | *F. orthocera* | 4.61 | 93.1 | 5.2 | 3.89 | 92.9 | 10.9 |
| *T–S–20* | *F. concolor* | 4.15 | 94.2 | 4.8 | 3.49 | 94.1 | 10.7 |
| *T–S–38* | *F.* sp. | 3.80 | 93.6 | 4.9 | 4.49 | 94.8 | 9.5 |
| *T–S–35* | *F. culmorum* | 4.40 | 93.5 | 4.1 | 4.02 | 93.5 | 8.4 |
| *T–S–16* | *F. Equiseti* | 3.63 | 93.2 | 8.3 | 5.25 | 91.5 | 7.3 |
| *T–S–26* | *F. avenaceum* | 3.65 | 92.5 | 6.9 | 4.67 | 93.6 | 7.2 |
| *T–S–18* | *F. solani* | 3.70 | 92.3 | 8.2 | 5.10 | 95.1 | 6.1 |
| *T–S–22* | *F. oxysporum* | 3.80 | 92.5 | 6.1 | 4.92 | 91.8 | 5.9 |
| *T–S–19* | *F. oxysporum* | 3.52 | 92.3 | 9.0 | 5.41 | 92.4 | 5.6 |
| *D–163* | *F.* sp. | 3.40 | 93.6 | 8.2 | 4.55 | 92.9 | 4.0 |
| *T–S–39* | *F.* sp. | 4.88 | 94.8 | 4.4 | 5.15 | 94.7 | 1.4 |

and relatively high lignin breakdown. At 40 days, however, the correlation between sugar and pH was not significant ($r = .07$). The correlation between lignin and pH was $-.78$, which indicated that now the high pH was associated with a low lignin breakdown. The partial correlation coefficient of $-.79$ shows that this relation was independent of the effect of sugar. The *Fusarium* cultures all brought about rapid fermentation of the sugar but they did not carry the lignin decomposition as far as certain *Alternaria* cultures. There was no correlation between the 20- and 40-day periods for any of the factors studied.

*Miscellaneous Soil Fungi*

A number of fungi belonging to different genera that may be found on decaying vegetable matter, or in soil, were tested on the calcium lignosulphonate media. They have been arranged in Table III in the order of those giving the greatest lignin breakdown at 20 days.

Since this is a heterogeneous group of organisms there are wide variations in the final pH of the culture medium and also in the amount of sugar fermented. The *Botrytis* and *Cephalothecium* species were both active in the fermentation of sugar and at the same time produced a low pH. The two cultures of *Aspergillus* fermented the sugar very completely in 10 days, but did not alter the pH greatly. As might be expected from an unrelated series of organisms, the coefficients of correlation between pH and sugar, pH and lignin, and lignin and sugar, did not reach significance except in the case of lignin decomposition and pH at 20 days, when there was some tendency for increased decomposition to be associated with high pH. However, the corre-

TABLE III

ANALYTICAL RESULTS FROM MISCELLANEOUS SOIL FUNGI GROWN ON CALCIUM LIGNOSULPHONATE MEDIA

| N.R.C. culture No. | Name | Incubation time, days | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10 | | | 20 | | |
| | | pH | Sugar fermented, % | Lignin decomposed, % | pH | Sugar fermented, % | Lignin decomposed, % |
| *T–G–7* | *Phoma* sp. | 4.25 | 48.4 | 5.2 | 4.91 | 97.6 | 12.3 |
| *C–66* | *Trichoderma* sp. | 4.20 | 71.0 | 9.3 | 4.30 | 97.9 | 10.1 |
| *T–G–72* | *Epicoccum purpurascens* | 4.10 | 14.6 | 4.2 | 3.50 | 63.8 | 9.8 |
| *L–125* | *Trichoderma* sp. | 6.55 | 73.8 | 1.4 | 7.18 | 95.6 | 9.7 |
| *D–61* | *Aspergillus* sp. | 5.88 | 95.5 | 3.7 | 5.81 | 96.3 | 8.4 |
| *T–G–63* | *Stemphylium* sp. | 3.93 | 45.7 | 4.1 | 3.75 | 56.1 | 8.4 |
| *T–G–16* | *Stemphylium* sp. | 4.28 | 34.6 | 4.8 | 4.38 | 96.9 | 8.0 |
| *T–G–51* | *Phoma vulgaris* | 3.99 | 45.2 | 2.1 | 3.80 | 65.6 | 7.0 |
| *T–G–86* | *Botrytis* sp. | 2.92 | 82.3 | 6.1 | 5.39 | 96.2 | 6.4 |
| *T–G–54* | *Phoma* sp. | 4.41 | 27.7 | 5.8 | 3.85 | 62.0 | 6.3 |
| *T–G–79* | *Cephalothecium* sp. | 2.80 | 95.1 | 6.8 | 3.48 | 98.1 | 6.1 |
| *T–G–65* | *Stemphylium* sp. | 4.11 | 11.3 | 2.8 | 3.15 | 84.7 | 6.0 |
| *L–37* | *Aspergillus niger* | 4.58 | 93.6 | 1.9 | 5.00 | 100.0 | 5.7 |
| *T–G–17* | *Stemphylium* sp. | 4.00 | 80.2 | 1.8 | 5.10 | 99.4 | 5.7 |
| *T–G–84* | *Cladosporium* sp. | 3.95 | 20.8 | 1.7 | 3.53 | 42.6 | 5.7 |
| *T–G–91* | *Stemphylium botryosum* | 3.48 | 78.9 | 4.2 | 3.39 | 91.1 | 5.6 |
| *T–G–48* | *Melanospora* sp. | 4.01 | 41.2 | 2.7 | 3.68 | 71.3 | 5.3 |
| *T–G–66* | *Stemphylium* sp. | 3.69 | 59.5 | 2.3 | 3.25 | 95.0 | 5.3 |
| *D–92* | *Botrytis* sp. | 3.65 | 2.7 | 0.7 | 4.30 | 30.3 | 5.2 |
| *D–2* | *Trichoderma* sp. | 4.30 | 37.5 | 3.0 | 3.90 | 100.0 | 4.3 |
| *T–G–87* | *Cephalothecium* sp. | 2.85 | 91.6 | 6.2 | 3.22 | 97.5 | 3.6 |
| *T–G–53* | *Phoma melina* | 4.25 | 19.9 | 3.4 | 4.05 | 38.8 | 3.1 |
| *H–2* | *Mucor racemosus* | 4.27 | 75.4 | 0.7 | 5.4 | 100.0 | 2.8 |
| *T–G–75* | *Botrytis* sp. | 2.95 | 88.0 | 2.5 | 4.40 | 96.2 | 2.8 |
| *T–G–73* | *Verticillium* sp. | 4.25 | 13.2 | 1.4 | 4.18 | 20.4 | 1.8 |
| *T–G–62* | *Chaetomium globosum* | 4.32 | 29.1 | 1.9 | 4.03 | 51.5 | 0.3 |

lation between the results at 10 and 20 days for these different factors was significant, being 0.48 for pH, 0.77 for sugar, and 0.44 for lignin. This means that given the analysis at 10 days it is possible to some extent to predict the trend at 20 days, particularly in the case of sugar. In this group of 26 different isolates from 13 different genera, the majority of the species used were not particularly promising as lignin decomposers.

## THE DECOMPOSITION OF CALCIUM LIGNOSULPHONATE BY WOOD DESTROYING FUNGI

In addition to common soil fungi, and species abundant on decaying vegetation that might hold promise for lignin breakdown, there remained the large group of fungi that attack and bring about various rots in wood. Thirty different species belonging to several genera were used to inoculate calcium lignosulphonate solutions of the same composition as previously used. Since these fungi generally grow much more slowly than the common moulds on

synthetic media, it was necessary to extend the growth period considerably, and analyses were made at 30- and 60-day intervals. The results are shown in Table IV.

TABLE IV

ANALYTICAL RESULTS FROM WOOD DESTROYING FUNGI GROWN ON CALCIUM LIGNOSULPHONATE MEDIA

| Division of Botany C.E.F. culture No. | Species | 30 days | | | 60 days | | |
|---|---|---|---|---|---|---|---|
| | | pH | Sugar fermented, % | Lignin decomposed, % | pH | Sugar fermented, % | Lignin decomposed, % |
| 8461 | *Poria ferrea* | 4.52 | 24.3 | 12.1 | 3.25 | 47.9 | 14.8 |
| 6893 | *Polyporus abietinus* | 4.12 | 37.4 | 14.2 | 4.10 | 63.7 | 13.6 |
| 8445 | *Fomes robustus* var. *tsugina* | 4.50 | 24.9 | 11.8 | 4.40 | 30.5 | 12.7 |
| 3445 | *Polyporus anceps* | 4.30 | 41.7 | 12.6 | 3.89 | 94.7 | 11.9 |
| 598 | *Poria subacida* | 3.95 | 31.4 | 11.0 | 3.95 | 42.6 | 11.9 |
| 8217 | *Trametes tenuis* | 4.32 | 23.7 | 12.5 | 3.95 | 33.0 | 11.7 |
| 7997 | *Polyporus tuberaster* | 3.90 | 37.0 | 9.8 | 3.73 | 71.4 | 10.7 |
| 7526 | *Polyporus compactus* | 4.32 | 31.6 | 8.6 | 4.05 | 54.7 | 10.5 |
| 8549 | *Polyporus resinosus* | 3.78 | 53.1 | 12.1 | 3.75 | 76.3 | 10.7 |
| 7403 | *Poria obliqua* (Sterile *Fomes igniarius*) | 4.23 | 34.1 | 6.9 | 3.88 | 59.3 | 9.0 |
| 8754 | *Poria Weirii* | 4.05 | 25.1 | 8.4 | 3.89 | 31.0 | 7.6 |
| 8049 | *Polyporus tulipiferus* | 3.95 | 56.5 | 7.8 | 3.89 | 81.3 | 6.2 |
| 9214 | *Fomes fomentarius* | 3.25 | 52.5 | 9.7 | 3.21 | 55.1 | 6.1 |
| 3580 | *Fomes Everhartii* | 4.33 | 41.5 | 4.7 | 3.95 | 86.6 | 5.6 |
| 9235 | *Polyporus ochroleucus* | 4.15 | 45.5 | 10.6 | 3.89 | 76.0 | 5.6 |
| 8415 | *Fomes annosus* | 3.85 | 47.9 | 11.0 | 3.95 | 85.6 | 5.1 |
| 5885 | *Polyporus pargamenus* | 4.20 | 27.3 | −4.1 | 4.00 | 51.9 | 4.9 |
| 7531 | *Polyporus pubescens* | 3.25 | 79.1 | 9.2 | 5.35 | 95.1 | 1.1 |
| 7120 | *Fomes pinicola* | 1.82 | 35.5 | −4.0 | 1.97 | 34.4 | −0.1 |
| | *Lenzites trabea* | 3.45 | 86.3 | 12.7 | 3.95 | 94.1 | −0.7 |
| 2163 | *Fomes Pini* | 4.28 | 32.9 | −2.9 | 3.92 | 69.2 | −0.7 |
| 8214 | *Fomes officinalis* | 1.85 | 29.2 | 0.0 | 1.71 | 41.3 | −1.0 |
| 8448 | *Poria ambigua* | 4.43 | 95.6 | 3.2 | 5.00 | 97.3 | −1.2 |
| 7340 | *Poria ferruginea-fusca* | 3.62 | 95.6 | 0.8 | 3.70 | 95.2 | −3.3 |
| 8282 | *Poria* sp. | 1.75 | 56.6 | −3.5 | 1.68 | 54.8 | −3.4 |
| 8787 | *Merulius lacrymans* | 3.39 | 24.4 | −3.2 | 2.13 | 58.7 | −3.4 |
| 8240 | *Polyporus oregonensis* | 3.69 | 98.2 | −1.5 | 3.81 | 84.5 | −3.6 |
| 8008 | *Fomes lobatus* | 3.81 | 55.5 | 2.1 | 5.00 | 94.5 | −3.8 |
| 8183 | *Polyporus versicolor* | 3.85 | 79.1 | 13.8 | 4.40 | 95.1 | −4.2 |
| 8287 | *Polyporus dichrous* | 4.49 | 2.97 | −4.7 | 4.60 | 17.5 | −6.9 |

The most outstanding difference in the effects brought about by wood destroying fungi as compared with soil fungi on calcium lignosulphonate media is the tendency for many of them to bring about an apparent increase of the amount of lignin in the solution. This was accentuated by a long growth period, but even at 30 days the effect was noticeable. Owing to the slow and scanty growth it is doubtful whether analyses at an earlier period would have yielded satisfactory information. It is worth noting that three cultures, *Fomes pinicola* (Swartz) Cooke, *Fomes officianalis* (Vill.) Fries, and *Poria* sp. gave very low pH values at both 30 and 60 days, indicating strong acid formation. With the exception of *Poria ambigua* Bres., *Poria ferruginea-fusca*

Karst., and *Polyporus oregonensis* (Murr.) Kauf. which utilized over 95% of the sugars in 30 days, these fungi were not very active sugar fermenters. Of the 30 species, about one-third decomposed over 10% of the lignin at the end of 60 days, and this was in most cases equally marked at 30 days, as indicated by the correlation $r = .69$ between the amount of lignin decomposed at 30 and 60 days. There was also a significant positive correlation for pH and for sugar between the 30- and 60-day periods. At 30 days there was a moderate but significant correlation between lignin breakdown and pH ($r = .39$). This relation no longer existed at the 60-day period ($r = .11$). At 60 days the greater the amount of sugar fermented the higher the pH became on the average as indicated by the correlation factor (.37).

Except when there was a high lignin breakdown, the precipitate of calcium lignosulphonate with $\beta$-naphthylamine was of the type described for the *Fusarium* cultures, which showed an apparent increase in lignin content after a 40-day growth period. By means of spectroscopic analysis it has been possible to study this effect in more detail and offer an explanation for this apparent increase in lignin. These observations will be presented in a later paper.

## Reproducibility of the Lignin Decomposition Results

Since the previous experiments were all done on a single culture of each of the organisms, it was thought advisable to repeat the growth of certain cultures in triplicate in order to confirm their lignin decomposing action. It has been found in previous work on fungous cultures that satisfactory repetition of results is often difficult to obtain. Consequently five cultures of *Alternaria* and two of *Fusarium* which gave the best decomposition of calcium lignosulphonate in the general experiments were repeated in triplicate. The growth period was prolonged to 40 days and the analyses carried out at 20 and 40 days. The 20-day analyses provided a comparison with the previous single growth experiment, and the 40-day period allowed further changes to be measured. The controls were in triplicate. The results are shown in Table V.

It will be seen from the comparisons in Table V that with the exception of *F. avenaceum* (Fr.) Sacc. and *Alternaria* sp. (*D*–96) the general growth as measured by the sugar removal was slower in the triplicates than in the single experiment. This effect is reflected in the lignin breakdown since it is correspondingly lower at the end of 20 days in the triplicates. However, at the end of 40 days' growth the triplicates and the single cultures compare very favourably. The agreement between the individual replicates in the experiment are satisfactory both for sugar and lignin loss. In *Alternaria* sp. (*L*–21) flasks that had poor growth and relatively lower sugar fermentation at 20 days, had a correspondingly low lignin breakdown, and although the fermentation of sugar was complete at 40 days, the lignin breakdown was not as great as in those cultures with better sugar utilization. In general, the mean of the triplicates compares favourably with the result of the single experiments. The variation between individual uninoculated controls was less than 0.5%.

TABLE V

COMPARISON OF SINGLE AND TRIPLICATE ANALYSIS OF *Alternaria* AND *Fusarium* CULTURES

| N.R.C. culture No. | Species | Original analysis, 20 days | | | Triplicate analysis, 20 days | | | Triplicate analysis, 40 days | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | Sugar fermented, % | Lignin decomposed, % | pH | Sugar fermented, % | Lignin decomposed, % | pH | Sugar fermented, % | Lignin decomposed, % |
| *D*–96 | *Alternaria* sp. | 4.01 | 49.33 | 16.80 | 3.65 | 49.4 | 9.9 | 3.13 | 97.5 | 16.6 |
| | | | | | 3.63 | 45.9 | 10.6 | 3.11 | 94.0 | 18.3 |
| | | | | | 3.58 | 50.1 | 11.9 | 3.17 | 86.6 | 15.8 |
| *L*–13 | *Alternaria* sp. | 3.87 | 46.24 | 15.06 | 4.00 | 13.2 | 3.6 | 2.60 | 89.0 | 12.8 |
| | | | | | 3.98 | 13.9 | 1.8 | 2.72 | 81.9 | 13.1 |
| | | | | | 4.00 | 7.5 | 3.3 | 2.83 | 73.2 | 13.4 |
| *L*–21 | *Alternaria* sp. | 3.35 | 77.90 | 16.98 | 3.57 | 42.8 | 9.1 | 3.01 | 97.5 | 13.0 |
| | | | | | 3.50 | 46.3 | 8.4 | 2.99 | 94.0 | 12.7 |
| | | | | | 3.70 | 31.6 | 5.2 | 3.98 | 93.3 | 8.3 |
| *T*–*G*–26 | *Alternaria* sp. | 3.70 | 76.81 | 13.62 | 3.78 | 45.1 | 7.2 | 3.25 | 83.6 | 13.1 |
| | | | | | 3.75 | 42.8 | 6.8 | 3.38 | 86.9 | 8.9 |
| | | | | | 3.69 | 53.8 | 6.2 | 3.30 | 95.1 | 12.3 |
| *T*–*S*–29 | *Alternaria* sp. | 3.78 | 86.76 | 13.84 | 3.71 | 45.7 | 7.9 | 3.05 | 96.9 | 9.2 |
| | | | | | 3.79 | 45.7 | 7.8 | 3.01 | 97.5 | 11.0 |
| | | | | | 3.70 | 45.7 | 7.3 | 3.00 | 96.3 | 11.4 |
| *T*–*S*–26 | *Fusarium avenaceum* | 3.65 | 92.50 | 6.93 | 3.55 | 97.4 | 9.5 | 3.10 | 95.7 | 11.7 |
| | | | | | 3.55 | 97.4 | 8.6 | 3.05 | 95.6 | 10.3 |
| | | | | | 3.53 | 97.4 | 11.6 | 3.07 | 96.9 | 10.3 |
| *T*–*S*–24 | *Fusarium culmorum* | 5.53 | 97.59 | 10.26 | 2.92 | 83.9 | 13.4 | 4.25 | 90.0 | 12.5 |
| | | | | | 3.10 | 87.2 | 13.1 | 3.95 | 90.6 | 10.2 |
| | | | | | 3.20 | 86.0 | 14.0 | 3.50 | 89.4 | 10.5 |

The above experiment shows that the agreement between individual cultures inoculated and grown for the same period and all analysed together is satisfactory. This, however, does not explain differences that arise when cultures identical in preparation and treatment are grown at different periods. In the above experiment the greatest differences are not between replicate cultures cultivated simultaneously but between replicate cultures inoculated and grown at different time intervals. In order to obtain more precise information on this point, cultures of each of the five organisms used in the previous experiment were grown without replication. Three days later an identical experiment was set up and inoculated. Both were analysed at the end of 20 and 30 days' growth. A statistical analysis of the chemical results was made. The results are shown in Table VI, and an analysis of variance is shown in Table VII.

It is apparent from Table VII that there was a satisfactory agreement for the pH and sugar determinations from replicate trials at different times. For the lignin determination, however, the major portion of the observed

TABLE VI

REPRODUCIBILITY OF RESULTS FROM TWO SEPARATE INOCULATIONS

| N.R.C. culture No. | Species | Analyses at 20 days | | | | | | Analyses at 30 days | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | | Sugar gm./litre | | Lignin gm./litre | | pH | | Sugar gm./litre | | Lignin gm./litre | |
| | | Inoculation | | | | | | Inoculation | | | | | |
| | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| *T-G-26* | *Alternaria* sp. | 4.12 | 4.08 | 8.5 | 2.0 | 24.9 | 22.1 | 3.83 | 5.52 | 1.2 | 0.4 | 22.6 | 22.9 |
| *T-S-24* | *Fusarium culmorum* | 5.40 | 5.33 | 0.8 | 0.5 | 25.3 | 22.7 | 5.45 | 5.50 | 0.7 | 0.4 | 24.6 | 24.0 |
| *T-S-26* | *Fusarium avenaceum* | 3.75 | 4.03 | 0.7 | 0.7 | 24.8 | 22.5 | 4.60 | 4.10 | 0.4 | 0.9 | 23.3 | 22.2 |
| *L-13* | *Alternaria* sp. | 3.98 | 3.98 | 11.7 | 10.0 | 25.7 | 21.7 | 4.38 | 3.38 | 1.4 | 1.1 | 22.9 | 22.1 |
| *D-96* | *Alternaria* sp. | 3.85 | 3.87 | 2.3 | 2.4 | 24.7 | 20.9 | 5.22 | 5.30 | 1.4 | 1.5 | 23.4 | 22.8 |
| | Control | 4.65 | 4.65 | 22.1 | 23.4 | 27.8 | 24.6 | 4.65 | 4.71 | 19.6 | 23.2 | 26.9 | 25.7 |

TABLE VII

ANALYSIS OF VARIANCE OF RESULTS FROM SEPARATE INOCULATIONS

| Source of variance | pH | | Sugar[1] | | Lignin | |
|---|---|---|---|---|---|---|
| | D.f. | Mean square | D.f. | Mean square | D.f. | Mean square |
| Replications | 1 | 0.0063 | 1 | 4.0861 | 1 | 21.4326** |
| Organisms | 5 | 1.0864* | 4 | 20.2154** | 5 | 6.6352** |
| Organisms × time | 5 | 0.3009 | 4 | 16.8855** | 5 | 0.4794 |
| Time of analysis 20 or 30 days | 1 | 1.0209 | 1 | 46.1472** | 1 | 0.8067 |
| Error | 10 | 0.2096 | 9 | 2.1116 | 11 | 0.9753 |

* *Exceeds mean square error, 5% level of significance.*

** *Exceeds mean square error, 1% level of significance.*

[1] *Degrees of freedom reduced because control sugars not included.*

variability occurred between the replicates made at different times and was attributable to factors beyond experimental control. The differences between organisms were consistent across all three criteria and there were also further differences shown by the sugar analysis in relation to time (20- and 30-day periods). The large variability between replicates shown in Table VII is explained by the consistently lower lignin content found in Replicate 2 for all organisms as compared with Replicate 1 at 20 days. The effect is not so pronounced at 30 days. The results for sugar fermentation show satisfactory agreement and demonstrate significant differences in the ability of the organisms to ferment sugar in 20 days. It was concluded from these experiments that the cultures could be satisfactorily reproduced at different times, but that extreme care is required to avoid bias in the chemical determination of lignin carried out on different occasions.

## Tannic Acid Reaction in Relation to Lignin Decomposition

Since analytical results on the breakdown of chemical lignin by a number of wood destroying fungi were available, it was decided to culture these species on tannic acid media and determine the correlation between the reaction and the amount of lignin decomposed. A medium containing 1.5% malt extract, 0.5% tannic acid, and 2.0% agar was used. The malt extract and agar were dissolved in 850 ml. of water, the tannic acid in 150 ml. and sterilized separately. The two solutions were mixed just before pouring the plates. The cultures were incubated for six days and the diameter of the colony and halo produced was measured. The different cultures were classified into six groups according to their reaction. These are as follows:

Class O: Negative, no colour change under or around inoculum.

Class I: No colour change under or around the fungous mat.

Class II: Diffusion zone, light to dark brown, under inoculum, visible from under side.

Class III: Diffusion zone light to dark brown under most of mat but not extending beyond the edge (halo, 1 to 5 mm.).

Class IV: Diffusion zone light to dark brown, extending a short distance beyond the edge of the mat or inoculum (halo, 6 to 10 mm.)

Class V: Diffusion zone very dark brown, extending considerably beyond the edge of mat or inoculum (halo, over 10 mm.).

The relation between this tannic acid reaction and calcium lignosulphonate decomposition found after 30- and 60-day growth periods is shown in Table VIII. The species have been arranged in three groups according to the intensity of reaction produced on the tannic acid medium.

The data in Table VIII were analysed statistically and it was found that between groups there was a slight but significant correlation between lignin breakdown and tannic acid reaction at 60 days but not at 30 days. If an organism is selected that belongs to Class IV or V with respect to tannic acid reaction, it may be predicted that its lignosulphonate decomposing ability will probably be better than if it were selected from the other classes. However, in view of the variation in lignin decomposition shown by species giving the same tannic acid reaction, the practical value of such a test would seem to be doubtful.

Most of the *Alternaria* and *Fusarium* cultures were also grown on tannic acid media and the reaction studied. Of the *Alternaria* cultures shown in Table I, one culture (*D*–96) fell into Class I, three cultures belonged to Class II, and the remainder were in Class IV or V. *D*–96 was one of the best lignosulphonate decomposing species tested. *Fusarium culmorum* and *F. Equisiti* (Corda) Sacc. gave a Class IV reaction and *F. solani* (Martius p.p.) Appel & Wollenw. and *F. oxysporum* Schlecht. a negative effect. There did not appear to be any correlation between this reaction and lignosulphonate breakdown.

TABLE VIII

TANNIC ACID REACTION AND CALCIUM LIGNOSULPHONATE DECOMPOSITION BY WOOD DESTROYING FUNGI

| Species showing reaction of Classes IV and V | Lignin decomposed, % | | Species showing reaction of Classes III and II | Lignin decomposed, % | | Species showing reaction of Classes O and I | Lignin decomposed, % | |
|---|---|---|---|---|---|---|---|---|
| | 30 days | 60 days | | 30 days | 60 days | | 30 days | 60 days |
| *Poria ferrea* | 12.1 | 14.8 | *Poria subacida* | 11.0 | 11.9 | *Polyporus tulipiferus* | 7.8 | 6.2 |
| *Polyporus abietinus* | 14.2 | 13.6 | *Trametes tenuis* | 12.5 | 11.7 | *Fomes Everhartii* | 4.7 | 5.6 |
| *Fomes robustus* var. *tsugina* | 11.8 | 12.7 | *Polyporus compactus* | 8.6 | 10.5 | *Fomes pinicola* | −4.0 | −0.1 |
| *Polyporus anceps* | 12.6 | 11.9 | *Polyporus resinosus* | 12.1 | 10.7 | *Poria* sp. | −3.5 | −3.4 |
| *Polyporus tuberaster* | 9.8 | 10.7 | *Poria Weirii* | 8.4 | 7.6 | *Merulius lacrymans* | −3.2 | −3.4 |
| *Poria obliqua* (Sterile *Fomes igniarius*) | 6.9 | 9.0 | *Fomes Pini* | −2.9 | −0.7 | *Polyporus dichrous* | −4.7 | 6.9 |
| *Fomes fomentarius* | 9.7 | 6.1 | *Poria ambigua* | 3.2 | −1.2 | | | |
| *Polyporus ochroleucus* | 10.6 | 5.6 | *Polyporus oregonensis* | −1.5 | −3.6 | | | |
| *Fomes annosus* | 11.0 | 5.1 | *Fomes lobatus* | 2.1 | −3.8 | | | |
| *Polyporus pargamenus* | −4.1 | 4.9 | *Polyporus versicolor* | 13.8 | −4.2 | | | |
| *Polyporus pubescens* | 9.2 | 1.1 | | | | | | |
| *Fomes officinalis* | 0.0 | −1.0 | | | | | | |
| *Poria ferruginea-fusca* | 0.8 | −3.3 | | | | | | |
| Mean | 8.06 | 7.03 | | 6.75 | 3.88 | | 1.4 | −0.3 |

## General Discussion

The determination of lignin in the calcium lignosulphonate media is more accurate than in media prepared from waste sulphite liquor. In the latter the lignin values as measured by the $\beta$-naphthylamine precipitation method gave erratic results in the controls, there being a tendency for the lignin content to undergo an apparent increase over a period of 30 days. This drift was independent of pH changes. However, the use of media prepared with isolated calcium lignosulphonate eliminated this difficulty almost entirely. The difficulty of obtaining consistent results in lignin determinations carried out at different intervals of time still remained, however, and care had to be exercised on this point.

The survey that has included species of soil fungi such as *Alternaria*, *Fusarium*, *Phoma*, and a few species from other genera and 30 different wood destroying fungi has shown that although most of the organisms tested are capable of decomposing calcium lignosulphonate, there is a wide variation in this respect even between closely related species. In general it appears that certain of the soil fungi are more promising than the wood destroyers.

The wood destroying fungi present more cultural difficulties, are much slower in their development, and after a long period of growth many of them

tend to give an apparent increase in lignin content as measured by the β-naphthylamine precipitation method. The *Alternaria* cultures gave the greatest lignin breakdown and in those species that were retested gave very consistent results. They grow rapidly and present few difficulties in culturing on synthetic media. Certain species of *Fusarium* were capable of giving good lignin decomposition in individual tests but gave erratic results when duplicate experiments were attempted at a later date. Of the other genera tested, *Trichoderma* and *Phoma* merit further study, and it must be recognized that tests on a number of species of *Mucor*, *Aspergillus*, *Penicillium*, *Stemphylium*, and *Verticillium* might disclose the same sort of variability found with cultures of *Alternaria* and *Fusarium*. It should be borne in mind in assessing the value of any particular fungus that all cultures were grown on a standard synthetic medium in which the various nutrients were by no means present in optimum concentrations for all these organisms. The fact that in each different class of organism studied the greatest lignin decomposition was in the range of 12 to 18% indicates that factors other than the organism itself are involved. These may include nutritional or environmental factors that are not optimum, or the production of certain toxic compounds during growth which cause staling. It has been suggested by other workers (4, 8) that the cessation of lignin decomposition results from the formation of ligno-protein complexes that are inhibitory or very resistant to further microbial action. It is possible that this mechanism may account for the cessation of decomposition of calcium lignosulphonate. The fact that the precipitate obtained with β-naphthylamine in old cultures is considerably greater than in corresponding uninoculated controls suggests that the calcium lignosulphonate has combined with some metabolic products, possibly protein. On the other hand, the fact that only 12 to 18% of the calcium lignosulphonate is available to the fungus may indicate that part of the molecule only is being split off, and when this is attained further breakdown does not occur. The actual fate of lignin in nature mitigates against this explanation, but Schwalbe and Ekenstam (6) have shown that the methoxyl groups are split off by microbial action. In order to throw light on these questions further work is being carried on using a spectrographic method of analysis. A detailed investigation of the nutritional factors concerned is also being studied, using one organism grown on sodium lignosulphonate medium.

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# STUDIES ON THE RELATION OF GROWTH RATE TO WOOD QUALITY IN *POPULUS* HYBRIDS[1]

By L. P. V. Johnson[2]


## Abstract

Experiments were conducted on the relation of growth rate to wood quality in a series of 43 hybrid and parental trees, which involved *Populus alba*, *P. grandidentata*, and *P. tremuloides*.

Fibres in fast growth annual rings were longer on the average than those in slow growth rings from the same tree. In single annual rings, fibres of early wood were shorter and thicker than those of late wood.

Average fibre diameter of individual trees was significantly correlated in a positive manner with growth rate, but the correlation between fibre length and growth rate was well below the level of significance.

Short, thick habit of growth was significantly correlated with high density of wood, but correlations between growth rate (in terms of annual increment in volume) and wood density were insignificant.

Experimental pulp and paper tests did not reveal any very striking differences in quality between fast growing hybrid and slow growing parental trees, although there remains some doubt as to the suitability of abnormally fast growth hybrid wood for some of the higher grades of soda pulp paper.

The general, and tentative, conclusion is that the investigation revealed nothing to indicate that rapid growth is seriously detrimental to wood quality.


## Introduction

It is a demonstrated fact that the growth rate of a tree affects the structure of the wood produced; for example, the more open structure of spring wood as compared to summer wood leads to the demarcation of annual rings. Therefore, since hybridity commonly introduces increased vigour of growth, the possibility of adverse wood characteristics arising from increased growth rate must be taken into account in the growing of hybrid trees.

The investigation herein reported, which is a part of a general program on forest tree breeding (2), represents an attempt to ascertain the degree to which the abnormally rapid growth (hybrid vigour) of *Populus* hybrids affects the quality of wood for pulping and other purposes. Fibre dimensions and specific gravity of the wood were used in general as the criteria of wood quality, but complete pulp and paper tests were also made on selected hybrid and parental trees.

## Materials and Methods

The materials studied are as follows:

| | |
|---|---|
| *P. alba* (*A*)* | 2 trees |
| *P. grandidentata* (*G*) | 6 trees |
| *P. tremuloides* (*T*) | 3 trees |
| *P. alba* × *P. grandidentata* $F_1$ (*A* × *G*) | 24 trees |
| *P. alba* × *P. tremuloides* $F_1$ (*A* × *T*) | 8 trees |
| Total | 43 trees |

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* *Letters in parentheses are the symbols used to designate these materials in the tabulated part of this paper.*

Data on ages and dimensions of these trees are given in Table I.

Of the younger trees, ages six to seven years, those of *P. alba* grew in gravelly soil near Hull, Que., and those of all other species on a gravelly site near Masson, Que. Of the older trees, ages nine years and older, *P. grandidentata*, Nos. 1 and 2, grew near the edge of a bush adjacent to the Masson site, and all others near the Aylmer Road, Hull, Que., in low gravelly soil. Apart from *P. grandidentata*, Nos. 1 and 2, all trees were sufficiently in the open to escape suppression by other trees.

The following measurements were made on each tree: diameter, determined at stump and breast heights; height, determined by a measuring stick for trees under 35 ft. and by an hypsometer for higher trees; age, determined by the number of annual rings in the core (extracted by a standard increment borer); specific gravity, determined in a 1½-in. core removed from the butt by a specially designed borer, at a height of 18 in. from the ground. All holes were plugged with creosoted cedar dowels in order to preserve the trees for future studies.

Wood samples from spring, middle, and summer regions of average growth annual rings (and, in certain trees, fast growth and slow growth annual rings) were macerated and fibre dimensions were determined microscopically. In selecting an average growth ring, primary consideration was given to the width of the ring rather than to the year in which the ring was produced. There may be some objection to this procedure since length of fibre may vary with age of ring. However, it was considered more important, in a study dealing with growth rate, to select rings that represent average growth rate rather than rings of standard age. As it happened, average growth rings tended to occur midway between centre and bark; in six-year–old trees, for example, the average growth ring was that representing the third or, more commonly, the fourth year's growth.

Wood was macerated by immersing for 48 hr. in a freshly combined mixture of 10% nitric acid and 10% chromic acid.

All samples were permanently mounted for microscopic observations. For the most part a modification of Zirkle's (4) mounting medium was used. The procedure is as follows:

(1) Prepare a mounting solution by mixing 4 parts glacial acetic acid, 1 part "Certo", 1 part corn syrup.

(2) Wash macerated material.

(3) Tease material into separate fibres in a drop of mounting solution on a slide.

(4) Apply cover glass and seal with asphalt varnish. (A series of trial slides that were left unsealed have remained in excellent condition for several months.)

Another method of permanent mounting which, though longer than the above, proved entirely satisfactory, is as follows:

(1) Wash macerated material.
(2) Pass through 70%, 95%, and two changes of absolute alcohol, allowing 10 min. in each.
(3) Tease fibres apart on slide.
(4) Mount in Canada balsam.

Microscopic observations were made with a 16 mm. objective and a 5× ocular (50× magnification). Fibre dimensions were determined with a 5.0 mm. ocular micrometer (with the optical system used, 5.0 mm. in the ocular corresponds very closely with 1.0 mm. on the field).

In all observations on fibre length, three counts of 10 fibres each were made. As may be seen in Table IV there was very little variation between counts, and it has not been considered necessary to subject this variation to statistical analysis. The fibre length data as given in Tables II and III represent the average of three 10-fibre counts for each section of the annual ring (e.g., early wood).

In all observations on fibre diameter one count of 10 fibres was made.

Tree volumes (under the bark) were calculated from logarithmic volume tables for poplar issued by the Dominion Forest Service.

Specific gravity determinations were made by: determining the volume of trimmed, soaked (3 hr.) cores (½ in. in diameter, 1½ in. long) by the water displacement method using a small specific gravity balance and weighing to the nearest 0.01 gm.; determining dry weight of the cores after drying overnight in an oven at 110° C.; and calculating specific gravity by dividing the volume in millilitres into dry weight in grams.

## Experimental Results

### 1. *Vigour Index*

The product of the average annual increments in height and in diameter, herein called the vigour index, is used to express the relative vigour of growth of the "uneven-aged"* trees of the present study (Table I). Had the trees been "even-aged"**, the average annual increment in volume $\left(\frac{\text{volume}}{\text{age}}\right)$ would have been a more accurate measure of vigour since volume tables take into account the taper of the trunk (under the bark). When dealing with a series of uneven-aged trees, however, the average annual increment in volume cannot be used since it is partly dependent upon the area of cambium which, as a function of the circumference, would be disproportionately greater in large trees. In the present study the use of average annual increment in volume as an index of vigour has been restricted to the 6-yr. age class (Table V).

The vigour indices are given in Table I and are repeated as one of the variables arranged for correlative studies in Table V. Correlation coefficients between the vigour indices and other variables are given in Table V and are discussed in Subsection 6 below.

* *Group comprising trees of different ages.*
** *Group comprising trees of the same age.*

TABLE I

GENERAL DATA ON AGE, SIZE, AND VIGOUR OF TREES, AND SPECIFIC GRAVITY

| Material | Age, yr. | Height, ft. | Diameter over bark, in. | | Volume, cu. ft. | Average annual increment | | Vigour index *AB* | Specific gravity |
|---|---|---|---|---|---|---|---|---|---|
| | | | Stump height (18 in.) | Breast height | | Height, ft. (*A*) | Diameter, in. (*B*) | | |
| *A*–1* | 6 | 20 | 3.8 | 3.3 | 0.75 | 3.3 | 0.63** | 2.10 | 0.459 |
| *A*–2 | 7 | 22 | 4.3 | 3.7 | 1.0 | 3.1 | 0.61 | 1.92 | 0.502 |
| *G*–1 | 17 | 34 | 3.2 | 2.8 | 0.84 | 2.0 | 0.19 | 0.38 | 0.369 |
| *G*–2 | 19 | 40 | 3.9 | 3.4 | 1.38 | 2.1 | 0.21 | 0.44 | 0.361 |
| *G*–3 | 16 | 46 | 5.4 | 4.6 | 2.76 | 2.9 | 0.34 | 0.98 | 0.430 |
| *G*–4 | 13 | 60 | 7.7 | 6.5 | 6.70 | 4.6 | 0.59 | 2.73 | — |
| *G*–5 | 19 | 76 | 13.6 | 11.9 | 22.33 | 4.0 | 0.76 | 3.04 | 0.413 |
| *G*–6 | 15 | 75 | 10.9 | 9.5 | 16.85 | 5.0 | 0.73 | 3.65 | — |
| *T*–1 | 7 | 25 | 3.6 | 3.1 | 0.79 | 3.6 | 0.51 | 1.82 | 0.437 |
| *T*–2 | 6 | 29 | 3.9 | 3.4 | 1.05 | 4.8 | 0.65 | 3.14 | 0.424 |
| *T*–3 | 6 | 29 | 3.8 | 3.3 | 0.99 | 4.8 | 0.63 | 3.04 | 0.363 |
| *A* × *G*–2 | 6 | 25 | 5.0 | 4.3 | 1.47 | 4.2 | 0.83 | 3.46 | 0.383 |
| –5 | 6 | 31 | 4.4 | 3.8 | 1.37 | 5.2 | 0.73 | 3.77 | 0.378 |
| –13 | 7 | 20 | 3.8 | 3.3 | 0.75 | 2.9 | 0.54 | 1.54 | 0.365 |
| –14 | 6 | 26 | 3.7 | 3.2 | 0.86 | 4.3 | 0.62 | 2.68 | 0.393 |
| –21 | 6 | 19 | 3.5 | 3.0 | 0.60 | 3.2 | 0.58 | 1.84 | — |
| –23 | 6 | 24 | 4.6 | 3.9 | 1.17 | 4.0 | 0.77 | 3.08 | 0.384 |
| –24 | 6 | 26 | 3.7 | 3.2 | 0.86 | 4.3 | 0.62 | 2.68 | 0.397 |
| –33 | 14 | 62 | 13.7 | 12.0 | 21.20 | 4.4 | 0.98 | 4.34 | 0.462 |
| –35 | 18 | 75 | 15.5 | 13.5 | 28.95 | 4.2 | 0.86 | 3.59 | 0.400 |
| –37 | 13 | 61 | 9.8 | 8.5 | 11.40 | 4.7 | 0.75 | 3.52 | 0.442 |
| –38 | 15 | 62 | 11.6 | 10.2 | 15.95 | 4.1 | 0.77 | 3.18 | 0.400 |
| –67 | 6 | 29 | 3.4 | 2.9 | 0.78 | 4.8 | 0.57 | 2.75 | 0.407 |
| –72 | 6 | 27 | 2.9 | 2.5 | 0.52 | 4.5 | 0.48 | 2.16 | 0.344 |
| –73 | 7 | 29 | 4.6 | 3.9 | 1.38 | 4.1 | 0.66 | 2.73 | 0.371 |
| –74 | 6 | 29 | 4.3 | 3.7 | 1.22 | 4.8 | 0.72 | 3.48 | 0.383 |
| –75 | 6 | 33 | 4.7 | 4.0 | 1.59 | 5.5 | 0.78 | 4.29 | 0.356 |
| –76 | 6 | 34 | 4.8 | 4.1 | 1.71 | 5.7 | 0.80 | 4.54 | 0.338 |
| –77 | 6 | 28 | 4.3 | 3.7 | 1.20 | 4.7 | 0.72 | 3.36 | 0.339 |
| –91 | 7 | 23 | 3.5 | 3.0 | 0.69 | 3.3 | 0.50 | 1.65 | 0.469 |
| –92 | 6 | 29 | 4.5 | 3.9 | 1.36 | 4.8 | 0.75 | 3.62 | 0.407 |
| –93 | 6 | 27 | 5.0 | 4.3 | 1.56 | 4.5 | 0.83 | 3.74 | 0.366 |
| –94 | 6 | 23 | 3.5 | 3.0 | 0.69 | 3.8 | 0.58 | 2.22 | 0.405 |
| –96 | 6 | 24 | 4.2 | 3.6 | 1.10 | 4.0 | 0.70 | 2.80 | 0.434 |
| –106 | 6 | 34 | 5.2 | 4.4 | 1.96 | 5.7 | 0.87 | 4.93 | 0.365 |
| *A* × *T*–1 | 6 | 24 | 4.5 | 3.9 | 1.18 | 4.0 | 0.75 | 3.00 | 0.424 |
| –2 | 6 | 22 | 2.9 | 2.5 | 0.47 | 3.7 | 0.48 | 1.76 | — |
| –4 | 6 | 17 | 2.1 | 1.9 | 0.28 | 2.8 | 0.35 | 0.99 | — |
| –5 | 6 | 21 | 3.9 | 3.4 | 0.82 | 3.5 | 0.65 | 2.28 | 0.440 |
| –6 | 7 | 20 | 2.6 | 2.3 | 0.38 | 2.9 | 0.37 | 1.06 | — |
| –12 | 9 | 55 | 7.1 | 6.1 | 5.52 | 6.1 | 0.79 | 4.83 | 0.355 |
| –19 | 7 | 24 | 3.3 | 2.9 | 0.67 | 3.4 | 0.47 | 1.61 | 0.364 |
| –20 | 7 | 27 | 3.8 | 3.3 | 0.95 | 3.9 | 0.54 | 2.08 | 0.347 |

* *See section on Materials and Methods for explanation of symbols.*

** *Based on diameter at stump height.*

### 2. *Fibre Dimension in Fast Growth and Slow Growth Annual Rings of the Same Tree*

Observations were made on the fibre dimensions of individual fast growth and slow growth annual rings obtained in a single core. This procedure permits a study of fibre development as related to variations in growth rate

## TABLE II

COMPARISON OF FIBRE DIMENSIONS IN FAST GROWTH AND SLOW GROWTH ANNUAL RINGS OF THE SAME TREE

| Material | Fast growth annual ring | | | | | | | | | Slow growth annual ring | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Width of ann. rings, mm. | Fibre length, mm. | | | | Fibre diameter, mm. | | | | Width of ann. rings, mm. | Fibre length, mm. | | | | Fibre diameter, mm. | | | |
| | | Early wood | Mid-season wood | Late wood | Av. | Early wood | Mid-season wood | Late wood | Av. | | Early wood | Mid-season wood | Late wood | Av. | Early wood | Mid-season wood | Late wood | Av. |
| *G*–4 | 11 | 0.96* | 1.01 | 1.03 | 1.00 | 0.026** | 0.029 | 0.024 | 0.026 | 3 | 0.83 | 0.96 | 1.02 | 0.94 | 0.027 | 0.024 | 0.022 | 0.024 |
| *G*–5 | 11 | 0.97 | 0.99 | 0.99 | 0.98 | 0.027 | 0.027 | 0.025 | 0.026 | 5 | 0.95 | 0.96 | 0.96 | 0.96 | 0.028 | 0.027 | 0.027 | 0.027 |
| *A* × *G*–2 | 13 | 0.78 | 0.95 | 0.98 | 0.90 | 0.027 | 0.026 | 0.026 | 0.026 | 3 | 0.52 | 0.53 | 0.63 | 0.56 | 0.025 | 0.025 | 0.025 | 0.025 |
| *A* × *G*–33 | 15 | 0.90 | 1.00 | 1.03 | 0.98 | 0.024 | 0.025 | 0.024 | 0.024 | 6 | 0.94 | 0.97 | 1.04 | 0.98 | 0.022 | 0.023 | 0.021 | 0.022 |
| *A* × *G*–35 | 11 | 0.92 | 1.01 | 1.00 | 0.98 | 0.030 | 0.029 | 0.028 | 0.029 | 3 | 0.72 | 0.76 | 0.84 | 0.77 | 0.026 | 0.026 | 0.026 | 0.026 |
| *A* × *G*–38 | 9 | 1.02 | 1.06 | 1.09 | 1.06 | 0.027 | 0.026 | 0.024 | 0.026 | 3 | 1.01 | 1.02 | 1.07 | 1.03 | 0.026 | 0.023 | 0.023 | 0.024 |
| *A* × *G*–93 | 12 | 0.77 | 0.98 | 0.97 | 0.91 | 0.032 | 0.030 | 0.030 | 0.031 | 4 | 0.76 | 0.79 | 0.79 | 0.78 | 0.034 | 0.034 | 0.033 | 0.034 |
| *A* × *G*–106 | 23 | 0.96 | 0.94 | 0.99 | 0.96 | 0.027 | 0.025 | 0.024 | 0.025 | 10 | 0.79 | 0.98 | 1.01 | 0.93 | 0.028 | 0.026 | 0.023 | 0.026 |
| *A* × *T*–12 | 14 | 0.70 | 0.99 | 1.05 | 0.91 | 0.026 | 0.022 | 0.021 | 0.023 | 4 | 0.88 | 0.89 | 0.91 | 0.89 | 0.024 | 0.023 | 0.022 | 0.023 |

* *Each figure on fibre length is based on 30 fibres, thus the average of the three samples from each ring is based on 90 fibres.*

** *Each figure on fibre diameter is based on 10 fibres, thus the average of the three samples from each ring is based on 30 fibres.*

caused solely by environmental factors—hereditary factors being constant in the somatic tissues of a single individual.

The data presented in Table II indicate that fibre length tends to be greater in fast growth annual rings. Of the nine trees examined, the fast growth annual rings of eight had longer fibres than had corresponding slow growth annual rings. In the remaining tree ($A \times G$–33) the fibre length was the same in both fast growth and slow growth rings. The difference in fibre length could be called striking only in three trees ($A \times G$–2, $A \times G$–35, and $A \times$ G–93). The analysis of variance method applied to fibre length data in fast and slow growth annual rings gave an $F$ value well above the 5% level of significance.

As regards fibre diameter, the data do not indicate any consistent relation between this character and rate of growth in individual annual rings. The $F$ value obtained was not significant.

3. *Fibre Dimensions of Early, Midseason, and Late Wood of the Same Annual Ring*

It is a well known fact that in trees the xylem elements produced in the spring differ in diameter (as shown by cross sections) from those produced later in the growing season. It is also generally thought that the rate of growth is relatively more rapid in the spring and early summer than in the later part of the growing season. This suggests a relation between cell size and growth rate and has led to observations on dimensions of fibre in wood produced in the early, middle, and late periods of the growing season, respectively. The results are given in Tables II and III.

The results may be stated briefly as follows: fibres produced in the spring have a strong tendency to be relatively thicker and shorter than those produced late in the growing season. Data in Tables II and III show that in the 27

TABLE III

COMPARISON OF FIBRE DIMENSIONS OF EARLY, MIDSEASON, AND LATE WOOD OF AN AVERAGE GROWTH ANNUAL RING

| Material | Fibre length, mm. | | | | Fibre diameter, mm. | | | |
|---|---|---|---|---|---|---|---|---|
| | Early wood | Mid-season wood | Late wood | Av. | Early wood | Mid-season wood | Late wood | Av. |
| *A*–1 | 0.84 | 0.97 | 0.87 | 0.89 | 0.026 | 0.025 | 0.026 | 0.026 |
| *G*–2 | 0.85 | 0.98 | 1.08 | 0.97 | 0.023 | 0.023 | 0.022 | 0.023 |
| *G*–5 | 0.93 | 0.98 | 0.97 | 0.96 | 0.029 | 0.029 | 0.026 | 0.028 |
| *T*–3 | 0.61 | 0.68 | 0.69 | 0.66 | 0.030 | 0.026 | 0.026 | 0.027 |
| $A \times G$–35 | 0.96 | 0.96 | 1.04 | 0.99 | 0.036 | 0.032 | 0.031 | 0.033 |
| –72 | 0.79 | 0.91 | 0.99 | 0.90 | 0.025 | 0.026 | 0.025 | 0.025 |
| –93 | 0.85 | 0.92 | 0.88 | 0.88 | 0.025 | 0.026 | 0.026 | 0.026 |
| –94 | 0.63 | 0.72 | 0.90 | 0.75 | 0.025 | 0.027 | 0.025 | 0.026 |
| –106 | 0.67 | 0.76 | 0.78 | 0.74 | 0.025 | 0.026 | 0.024 | 0.025 |

NOTE: *The footnote to Table II applies also to Table III.*

annual rings (nine each of fast, slow, and average growth rings) examined, fibres of early wood were shorter than those of late wood, those of midseason wood being, in general, of intermediate length. The *F* value (analysis of variance) obtained for fibre length in early, midseason, and late wood attained the 1% level of significance for data in each table. In average diameter, fibres of early wood were greater than those of late wood in 20 out of 27 rings, with diameters equal in six rings—there being only one ring in which late wood fibres were of greater diameter. Here again fibres of midseason wood tended to be intermediate. The *F* value obtained for fibre diameter in early, midseason, and late wood attained the 1% level of significance for combined data of fast and slow growth annual rings (Table II), but was not significant for average growth rings (Table III).

4. *Fibre Dimensions of Hybrids and Parents of Different Growth Rates*

From observations on fibre dimensions in early, midseason, and late wood (Tables II and III) it was found that the fibres in midseason wood were, in general, intermediate in dimensions and corresponded fairly closely to the average for the annual ring concerned. Therefore, in making observations on the relatively large number of remaining trees it was considered sufficient to use only the midseason wood.

In Table IV, data are given on the average length and average diameter of fibres, taken from the midseason part of average growth annual rings, of a series of 43 hybrid and parental trees which differ markedly in vigour of growth. The variability between counts on fibre length in a given annual ring is very slight, the greatest difference being in *G*–3 where counts ranged from 0.88 to 0.97 mm. The standard error is 0.0103. Variability in vigour indices is much greater, however, being from 0.38 to 3.65 among the parents and from 0.99 to 4.93 among the hybrids.

These data are included in the correlative studies described in Subsection 6.

5. *Specific Gravity Studies*

Specific gravity determinations were made on cores of wood (½ in. in diameter) from the trunks (18 in. above ground) of 37 hybrid and parental trees. Results are given in Table I.

Since the removal of large cores might prove injurious, an attempt was made to establish a relation between the specific gravity of core wood and of wood from typical lower branches of the same tree. The coefficient of correlation (*r*) calculated for the data obtained fell well below the 5% level of significance (Table V). It was concluded therefore that the specific gravity of branch wood and that of trunk wood of the same tree are not sufficiently related to permit the substitution of the former for the latter in the present study.

Specific gravity determinations were also made on samples (disks) taken at 4-ft. intervals along the trunks of a number of trees that were cut down. The following data, which represent the specific gravity of successive disks taken at 4-ft. intervals commencing at the base, are typical of the results

TABLE IV

FIBRE DIMENSIONS OF HYBRIDS AND PARENTS OF DIFFERENT GROWTH RATES

| Material | Vigour index | Midseason wood of average growth ring | |
|---|---|---|---|
| | | Average* fibre length, mm. | Average** fibre diameter, mm. |
| *A*–1 | 2.10 | 0.97 ± 0.01 | 0.025 |
| *A*–2 | 1.92 | 0.77 | 0.027 |
| *G*–1 | 0.38 | 0.95 | 0.024 |
| *G*–2 | 0.44 | 0.98 | 0.023 |
| *G*–3 | 0.98 | 0.93 | 0.026 |
| *G*–4 | 2.73 | 1.02 | 0.028 |
| *G*–5 | 3.04 | 0.98 | 0.029 |
| *G*–6 | 3.65 | 0.98 | 0.029 |
| *T*–1 | 1.82 | 0.74 | 0.026 |
| *T*–2 | 3.14 | 0.68 | 0.024 |
| *T*–3 | 3.04 | 0.68 | 0.026 |
| *A* × *G*–2 | 3.46 | 0.88 | 0.026 |
| –5 | 3.77 | 0.78 | 0.038 |
| –13 | 1.54 | 0.83 | 0.026 |
| –14 | 2.68 | 0.97 | 0.026 |
| –21 | 1.84 | 0.85 | 0.023 |
| –23 | 3.08 | 0.93 | 0.027 |
| –24 | 2.68 | 0.95 | 0.031 |
| –33 | 4.34 | 0.94 | 0.024 |
| –35 | 3.59 | 0.96 | 0.032 |
| –37 | 3.52 | 0.97 | 0.028 |
| –38 | 3.18 | 1.11 | 0.033 |
| –67 | 2.75 | 0.90 | 0.026 |
| –72 | 2.16 | 0.91 | 0.026 |
| –73 | 2.73 | 0.93 | 0.028 |
| –74 | 3.48 | 0.82 | 0.031 |
| –75 | 4.29 | 0.92 | 0.030 |
| –76 | 4.54 | 0.94 | 0.028 |
| –77 | 3.36 | 0.82 | 0.037 |
| –91 | 1.65 | 0.81 | 0.023 |
| –92 | 3.62 | 0.93 | 0.034 |
| –93 | 3.74 | 0.92 | 0.026 |
| –94 | 2.22 | 0.72 | 0.027 |
| –96 | 2.80 | 0.86 | 0.026 |
| –106 | 4.93 | 0.76 | 0.026 |
| *A* × *T*–1 | 3.00 | 0.93 | 0.026 |
| –2 | 1.76 | 0.93 | 0.029 |
| –4 | 0.99 | 0.75 | 0.022 |
| –5 | 2.28 | 0.94 | 0.028 |
| –6 | 1.06 | 0.90 | 0.031 |
| –12 | 4.83 | 0.95 | 0.030 |
| –19 | 1.61 | 0.85 | 0.030 |
| –20 | 2.08 | 0.80 | 0.027 |

* *Average based on three counts of 10 fibres each.*

** *Average based on one count of 10 fibres.*

obtained; *G*–2: 0.361, 0.355, 0.344, 0.326, 0.338, 0.352, 0.342, 0.360, 0.373, 0.380; *A* × *G*–106: 0.365, 0.306, 0.293, 0.293, 0.275, 0.280, 0.286, 0.327.

These results show that specific gravity is relatively high at the base, gradually becoming lower until a height of 15 to 20 ft. is reached, and then gradually increasing with each succeeding interval to the top of the tree.

TABLE V

CORRELATION COEFFICIENTS ($r$) CALCULATED FOR DIFFERENT COMBINATIONS OF VARIABLES

| Combination of variables | Age group | Number of trees | $r$ |
|---|---|---|---|
| Average annual increment in height with: | | | |
| average annual increment in diameter | All-age | 43 | .75** |
| vigour index | All-age | 43 | .92** |
| specific gravity | All-age | 37 | −.28 |
| average length of fibre | All-age | 43 | −.00 |
| average diameter of fibre | All-age | 43 | .43** |
| Average annual increment in diameter with: | | | |
| vigour index | All-age | 43 | .92** |
| specific gravity | All-age | 37 | .06 |
| average length of fibre | All-age | 43 | .12 |
| average diameter of fibre | All-age | 43 | .34* |
| Vigour index with: | | | |
| height/diameter | All-age | 43 | −.43** |
| volume/age | 6-yr. | 24 | .59** |
| specific gravity | All-age | 37 | −.16 |
| average length of fibre | All-age | 43 | .09 |
| average diameter of fibre | All-age | 43 | .40** |
| Height/diameter with: | | | |
| specific gravity | All-age | 37 | −.41** |
| average length of fibre | All-age | 43 | −.04 |
| average diameter of fibre | All-age | 43 | −.14 |
| Volume/age with: | | | |
| specific gravity | 6-yr. | 21 | −.22 |
| average length of fibre | 6-yr. | 24 | .01 |
| average diameter of fibre | 6-yr. | 24 | .27 |
| Specific gravity with: | | | |
| average length of fibre | All-age | 37 | −.02 |
| average diameter of fibre | All-age | 37 | −.25 |
| Average length of fibre with: | | | |
| average diameter of fibre | All-age | 43 | .17 |
| Core branch (specific gravity) | All-age | 37 | .26 |

* *Denotes attainment of 5% level of significance.*
** *Denotes attainment of 1% level of significance.*

### 6. *Correlation Between the Different Variables under Study*

The variables used for correlative studies include primary observational factors, such as tree dimensions, fibre dimensions, and specific gravity, and also secondary factors, such as vigour index, height–diameter ratio, and volume–age ratio.

Table V gives the values of the correlation coefficient ($r$) calculated for 24 pairs of variables, together with data on the numbers and age grouping of trees involved.

There is a very high correlation between average annual increments in height and a diameter, which was to be expected. The correlation coefficients between these factors and the vigour index, which is a function of them, are, of course, also high.

Fibre diameter is positively correlated with average annual increments in height (1% level of significance) and in diameter (5% level), and with vigour index (1% level).

It will be noted that the values of $r$ for fibre length in corresponding combinations are very low. This means that as growth rate is increased the diameter, but not the length, of fibres is significantly increased.

It is important to note how this increase in fibre diameter affects values of $r$ where specific gravity is concerned. It is found that $r$ has a negative value in all combinations, except that with average annual increment in diameter of trunk, but only in the combination with the height–diameter ratio does it reach significance (1% level). This means that as trees tend to become tall and slender (as opposed to short and thick) the specific gravity of the wood tends to decrease.

The remaining significant correlation involves the two general measures of growth rate, vigour index, and volume–age ratio. The value of $r$ is well above the 1% level of significance. As indicated in Subsection 1 above, the volume–age ratio is a more desirable measure of growth vigour than the so-called vigour index. However, since it was necessary to use the vigour index it is reassuring to know that it is highly correlated with the volume–age ratio.

7. *Experimental Pulp and Paper Tests of a Slow Growing Parent and a Fast Growing Hybrid*

It was considered important to investigate the differences in pulp (soda) and paper quality that might be found in wood samples derived from a relatively slow growing parent and from an abnormally rapid growing hybrid. Accordingly, entire trunks of two such trees were sent to the Pulp and Paper Division, Forest Products Laboratory, Montreal, for testing. The report of the Laboratory, which includes comparisons with commercial poplar pulp, is summarized and discussed in the following paragraphs.

*(a) Description of Materials*

The trees used in the pulp and paper tests were also used in the general studies, the *P. grandidentata* parent being *G*–2 and the *P. alba* × *P. grandidentata* hybrid being *A* × *G*–106. The growth of *G*–2 had been considerably suppressed by surrounding bush and at the time of cutting, when it was 19 years old, it was 40 ft. in height and 3.9 in. in diameter. The tree, *A* × *G*–106, grew near *G*–2 but under less competitive conditions; it grew very rapidly being 34 ft. in height and 5.0 in. in diameter at six years of age. For further data on *G*–2 and *A* × *G*–106 see Tables I to IV inclusive.

It was considered that, since these trees differed so greatly in growth rate, the tests should indicate whether any marked peculiarities in pulp or paper quality might exist in the wood of abnormally rapid growing poplar hybrids.

*(b) Chemical Analysis of the Wood*

No appreciable difference in chemical composition was indicated by wood analysis. *A* × *G*–106 was slightly higher than *G*–2 in lignin, but was consequently, somewhat lower in cellulose.

*(c) Screen Classification of Bleached Soda Pulp*

The screen classification showed that *G*–2 compared very closely with commercial poplar pulp in fibre length in both beaten and unbeaten conditions. The fibres of *A* × *G*–106, however, were shown to be appreciably longer than those of either *G*–2 or average commercial pulp; but not longer, however, than some of the longer-fibred commercial samples of poplar pulp.

*(d) Strength of Unbeaten and Unbleached Soda Pulp*

*A* × *G*–106 proved to be appreciably the stronger, both in slush and air-dry pulp, for burst factor and breaking length (length at which strip breaks of its own weight); it also gave a much higher percentage of stretch. In *G*–2 the bulk in ml. per gm. was considerably greater. In tear ratio the two samples were about equal.

*(e) Strength of Beaten and Bleached Soda Pulp*

Tappi beater tests (various periods of beating from 5 to 60 min.) of bleached soda pulp showed that *A* × *G*–106 was consistently stronger than *G*–2 or commercial pulp for burst factor and breaking length, and gave consistently higher percentages of stretch. At the start of the beating, *A* × *G*–106 gave a rather high tear ratio but after a few minutes of beating it registered a somewhat lower value than the other two samples. In bulkiness, *A* × *G*–106 was considerably below the other two samples for all beating periods.

*(f) Optical Properties*

Commercial poplar pulp was consistently better than that of *G*–2 or *A* × *G*–106 in brightness, reflectance, and printing opacity of the paper. This superiority was slight at the beginning of the beating period but became considerable after 60 min. The paper from *G*–2 was slightly better than that from *A* × *G*–106 in these optical qualities.

*(g) Discussion Regarding the Suitability of A* × *G–106 for Pulp Manufacture*

Before discussing the results it must be recognized that the present pulping tests do not provide an adequate basis for positive conclusions. Obviously, portions of several trees rather than a single trunk would be necessary to provide a representative sample, either of the hybrid or the parent. Further, in the present test the age of the hybrid tree was less than one-third that of the parent and probably less than one-fifth that of trees used in commercial pulping.

However, until better data are available, there are a few things that may be said with some safety about the present results.

For soda pulp paper, the pulp of *A* × *G*–106 appears to have one main peculiarity that might cause discrimination against it—that is, the condition of being stronger and less bulky than the pulp of ordinary mill-run poplars. Shorter, weaker fibres give the pulp a greater bulkiness, which in turn gives the desired formation and printing quality to the higher-grade soda pulp papers. It is possible that the pulp from trees such as *A* × *G*–106 might not meet the standards demanded for these papers.

It must be remembered, however, that a large part of the poplar wood pulped in Canada is utilized, in the sulphite process, as a mixture with spruce and balsam fir. In sulphite pulp, unlike soda pulp, a long, strong fibre is desired, and the proportion of poplar wood that may be mixed with the other pulpwoods is determined by the degree of weakening occasioned by the shorter poplar fibres. It follows, therefore, that if a rapid growing hybrid poplar had longer fibres than ordinary poplars, a correspondingly greater proportion of its wood could be mixed with other pulpwoods in the manufacture of sulphite pulp. This would lead to a greater demand for poplar pulpwood.

It may be, therefore, that there is a place in the pulp industry for both short- and long-fibre types of poplar.

It should be noted that results of the present studies (Subsection 6) do not establish any marked relation between growth rate and fibre length in a series of 43 trees.

## General Discussion and Conclusions

In the hybrid poplars under study, rapid growth is highly desirable—provided wood quality is not adversely affected. Therefore, to know the true commercial value of rapid growth (hybrid vigour) the relation between growth rate and wood quality must be known.

The data on fibre length in individual fast and slow growth annual rings from the same tree show that there is a marked tendency for fast growth rings to have the longer fibres. On the other hand, data on seasonal wood of single annual rings demonstrate unmistakably that early season wood has shorter fibres than late season wood. If, as seems to be the accepted view, wood is laid down faster in the early part of the growing season, these two types of data are in opposition with respect to the implied relation between growth rate and length of fibre produced. In the data from individual trees, statistically significant relation between fibre length and growth rate could not be demonstrated.

From the practical point of view, the conclusion from studies on fibre length must be: that it has not been possible to prove the existence of any consistent relation between fibre length and growth rate that might be reflected in the wood quality (as affected by fibre length) of rapid growing trees. This is in agreement with the results of a study on white spruce reported by Lee (3) who concluded that rate of growth had little effect on fibre length.

Data on fibre diameter from individual trees show a highly significant positive correlation with growth rate. Data from seasonal wood of single annual rings demonstrate a marked tendency for fibres of early season wood to be of larger diameter than those of late season wood. If it is assumed that growth is fastest in the spring, this result is in agreement with results from individual trees.

Since there would be less cell wall material per unit volume in tissue in which the fibres are relatively large in diameter, there is reason to expect that large diameter fibres would lead to a reduction in specific gravity of the

wood. In the present study, however, this relation was not strong enough to produce significant correlation coefficients when specific gravity was paired with either fibre diameter or growth rate. In so far as the present study is concerned, therefore, it can only be said that a relation between fibre diameter and growth rate was established, but that no definite information was obtained on how this relation might be reflected in the wood density of rapid growing hybrids.

In this connection, it is useful to cite the recent work of Hale and Prince (1) in which it was found that rapidly grown wood of spruce and balsam fir was of lower density than slowly grown wood. In their summary these authors make a statement that has a direct bearing on the practical aspects of the present discussion: ". . . although rapidly grown wood . . . is light in weight and the slowly grown wood relatively heavy, the rate of wood production of rapidly grown trees on a basis of total weight far exceeds the amount produced by slow growing trees. Therefore, from the point of view of the paper manufacturer, who requires large quantities (by weight) of wood produced in the shortest practicable time, fast growing trees will be essential, and extremely slow growing stands, however desirable the quality of their wood, will tend more and more to be uneconomical for pulpwood production."

The results of the pulp and paper tests have already been discussed at considerable length, and it remains merely to state that the tentative conclusion reached was that there appeared to be good reason to believe that rapid growing hybrid poplars could be utilized to good advantage either as soda or sulphite pulp or both. As a matter of fact, it may be possible for the breeder to produce long- and short-fibred trees for utilization as sulphite and soda pulp, respectively.

The general conclusion, which must be a tentative one, to be drawn from these studies is that nothing has been found that would support a contention that abnormally rapid growth is seriously detrimental to wood quality. For the time being, therefore, breeding work on the production of rapid growing forest trees will proceed with some assurance that rapid growth and good quality wood are not incompatible. It is hoped that further assurance in this connection may be forthcoming from future experiments.

## Acknowledgments

The author gratefully acknowledges the assistance rendered by the Forest Products Laboratories of the Dominion Forest Service; by Dr. J. W. Hopkins, Statistician at the National Research Laboratories; and by Lieut. R. A. Packman, formerly of Ontario Agricultural College.

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# THE DEVELOPMENT AND STRUCTURE OF THE CONIDIA OF *ERYSIPHE POLYGONI* DC. AND THEIR GERMINATION AT LOW HUMIDITY[1]

By Harold J. Brodie[2] and C. C. Neufeld[3]


## Abstract

The germination of the conidia of *Erysiphe Polygoni* DC. takes place through a range of relative humidity from approximately zero to 100% and, therefore, independently of the moisture content of the surrounding atmosphere. In germinating thus, they differ from the spores of some other erysiphaceous fungi and of non-erysiphaceous fungi in general.

In *E. Polygoni*, the conidium is cut off from the conidiophore by a ring of wall material which is added to inwardly until a perforate disk is formed. Later, the pore is closed and the mature conidium remains attached to its conidiophore only by a minute papilla. The conidia have never been observed to germinate *in situ*, and they are passively discharged.

The conidium wall is relatively impervious to water, stain passing into the spore only at the papillate end. Assuming the wall to be relatively impervious to gases also, an explanation is offered for the mechanism of germination of the conidia when they are dislodged from their conidiophores and allowed to fall on dry slides. The papilla provides a permeable spot in the spore wall. It is not exposed until after the spore has been detached. Upon exposure to air, the papilla allows carbon dioxide to pass out from the protoplast and oxygen to pass in, causing respiration and other germination processes to begin.

Evidence in support of this suggestion is presented. When freshly detached conidia were held in an atmosphere containing 10% carbon dioxide, germination was checked. These conidia germinated perfectly when removed from the carbon dioxide. Germination was similarly checked by holding the spores in an atmosphere of nitrogen.

No shrinkage of the conidia during germination was observed, but shrivelling and collapse take place when death is imminent.


## Introduction

In 1936, Yarwood (23) stated that the conidia of *Erysiphe Polygoni* DC. and certain other powdery mildews are capable of germinating at low relative humidity, even approaching zero.

It has been recognized that spores of fungi in general germinate best under conditions of high humidity and that, in many instances, they must be in actual contact with water. Yarwood's work, therefore, presented a problem of paramount importance, the solution of which might be expected to throw light upon the epidemiology and control of the powdery mildews and upon their physiology. Yarwood did not explain why mildew conidia are able to tolerate low humidity, and no confirmation of his observations has been offered up to the present.

The senior author, in 1937, carried out preliminary experiments with conidia of *E. Polygoni* taken from cabbage leaves. This work was not published, but

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it showed clearly that Yarwood had not been mistaken: a high percentage of germination of conidia was obtained under conditions of extremely low humidity. Encouraged by this finding, the writers undertook a further investigation of the peculiar behaviour of the mildew conidia in an effort to corroborate Yarwood's work and to discover why mildew spores tolerate low humidity.

## Historical

Prior to the publications of Yarwood (22, 23), there are few references in the literature containing data sufficiently relevant to the present problem to warrant detailed review in this paper.

Early workers concerned themselves chiefly with the taxonomy and morphology of the Erysiphaceae. Regarding the germination of conidia in the Erysiphaceae, the brothers Tulasne (20) merely mention that germinating spores were seen frequently in the material examined.

Neger (16), in 1902, studied the germination of mildew spores under different environmental conditions and showed that certain characteristics are typical of the germ tubes of each species. Each of these characteristics (length of germ tube, manner of branching, etc.) varies over a wide range and is affected by light and temperature. Similar views were expressed by Hammarlund (12) and Corner (8).

It has been observed repeatedly that the conidia of powdery mildews germinate only very scantily in water. Among those who have reported this fact in a large number of mildew species are Neger (16), Foëx (10), Sawada (18), Woodward (21), Graf-Marin (11), and Corner (8). According to Graf-Marin, Corner, and others, any germ tubes that are able to make their appearance in water may be longer than those produced in moist air. Sawada (18) reported that the conidia of some species of *Phyllactinia* and *Uncinulopsis* are killed by immersion in water.

As reported by the workers mentioned above, the optimum temperature for the germination of most powdery mildew conidia is about 25° C. *Uncinula necator* (Schw.) Burr. (*Oidium Tuckeri* Berk.) (24) and *Sphaerotheca pannosa* (Wallr.) Lev. (12) are exceptional, the optimum temperatures of germination being 25° to 28° and 30° C., respectively.

A considerable amount of investigation has been reported on the process of the development of the conidia. Brief reference to this work is made for comparison with the studies on the formation of conidia in *E. Polygoni* described in this paper.

It has been pointed out by Beeley (2), Blumer (5), and Hammarlund (12), that dry conditions favour the development of powdery mildew mycelium and encourage the production of conidia.

Massee (15) stated that the liberation of the conidia of *Sphaerotheca Humuli* (DC.) Burr. on vegetable marrow occurs chiefly at night. Hammarlund (12) reported that, in *Erysiphe communis* Auct. Amer. (*E. Polygoni*), from one to six

conidia are developed on each conidiophore each day and that the conidia are forcibly discharged.

In species that produce their conidia in chains, Foëx (9) showed that the basal cell and the one above it divide to produce the conidia (*S. Humuli*). Blumer (4) demonstrated that in *Erysiphe Cichoracearum* DC. and *Sphaerotheca* spp., the basal cell is the one from which conidia are formed.

Hammarlund (12) stated that, in *E. communis*, humidity affects the length of the chain of spores produced. In this fungus there is usually only one conidium formed at a time on each conidiophore. However, under moist conditions, chains of spores may be formed, the length of the chain increasing as the humidity increases.

In 1936, Yarwood (22) described, in *E. Polygoni* on red clover, a diurnal cycle which is manifest in the maturation of the conidia and their germinability. Each conidiophore forms one conidium per day and the spore is liberated passively about noon. Yarwood obtained the highest percentage of germination of mildew conidia when they were removed from clover from midday to four o'clock. Germinability decreased with the onset of darkness and reached a minimum in the early morning.

It was shown that light has a definite stimulatory effect upon germination: conidia collected during the high phase of the germination cycle germinated almost as well in darkness as in light, whereas germination of conidia collected in the low phase was greatly stimulated by light. In field experiments, inoculations with clover mildew spores made during the light portion of the day were more successful than inoculations made at night.

Recently, Childs (7) has demonstrated a similar cycle for a number of mildews including several that produce their spores in chains.

Working with *E. Polygoni* from various host plants, *E. Cichoracearum* from sunflower, and *S. pannosa* from rose, Yarwood (23) found that, at 22° C., germination of mildew conidia was good at relative humidities ranging from 100% to approximately zero, in many experiments the percentage germination at zero being as high as at 100%. Although as much as 65% germination was observed at zero relative humidity, the conidia were found to have shrivelled and died at the end of 30 hr., whereas at higher humidity a much smaller proportion shrivelled. In other words, the conidia tolerated low humidity to the extent of being able to germinate but ultimately more of them were injured by dry air than by moist. The tolerance of low humidity was also shown to decrease with increase of temperature.

Yarwood stated that the volume of the conidia decreases as much as 24% during germination. In contrast, conidia of several other fungi that did not germinate without being in actual contact with water increased greatly in volume during germination.

In field experiments, best infection of clover, bean, cabbage, barley, cantaloupe, *Delphinium*, and mustard was obtained when the inoculation was made at low humidity.

Yarwood's conclusion may be quoted: "Though, under certain conditions, better development of *E. Polygoni* has resulted under conditions of low rather than those of high humidity, the writer believes that high humidity is not, in itself, markedly injurious to the powdery mildews studied .... Rather, the writer believes that the forms studied can develop luxuriantly over a wide range of relative humidities and that they are especially well adapted, in contrast with most parasitic fungi, to very dry atmospheric conditions."

Not all species of powdery mildew tolerate low humidity. Yarwood found that *E. Polygoni* from mustard, *E. graminis* DC. from barley, *E. Cichoracearum* from sunflower, and *S. pannosa* from rose, were considerably less tolerant than other species and strains tested.

Berwith (3) studying *Podosphaera leucotricha* (E. & E.) Salm., the apple powdery mildew, claimed that high humidity is necessary for the germination of the conidia and the infection of apple seedlings, while Hashioka (13) showed that, in Formosa, the conidia of *Sphaerotheca fuliginea* (Schlecht.) Poll. on cucurbits do not germinate under conditions of low humidity. He believed, however, that the most abundant formation of conidia occurs at low humidity.

From the evidence presented above, it may be concluded that at least certain members of the Erysiphaceae are remarkable in that their conidia possess the ability to germinate under conditions of low humidity, in this respect being unlike other fungi. So far as the writers are aware, no explanation for this peculiar characteristic of the powdery mildews has been offered. The results of the investigations recorded in this paper throw some light upon the problem, although they cannot be said to present its complete solution.

## Materials and Methods

The organism most extensively used was *Erysiphe Polygoni* DC., obtained from knotweed (*Polygonum aviculare* L.) on the Fort Garry campus of the University of Manitoba during the summers of 1939 and 1940. Other mildew species were also examined; they will be mentioned later.

The mildewed leaves were gathered at midday when, as shown by Yarwood (22), the spores were found to exhibit maximum germinability. Germination tests were carried out immediately after collection, the conidia being placed in diffuse light in the laboratory at a temperature of 20° to 23° C. Clean cover slips were placed at the bottom of a large crock, and mildewed leaves were shaken to allow spores to settle on the cover slips assuring a uniform distribution.

Large Petri dishes sealed with vaseline served as chambers to provide a range of relative humidity from zero to 100%. Each dish was half-filled with a saturated salt solution according to the directions given by Spencer (19, pp. 67, 68). The cover slips bearing spores were supported above the solutions on glass rings. It was thought that, even in the presence of a humidity-regulating solution, the air in a small chamber might not be uniform as to

its moisture content. Yarwood placed the Petri plates on an incline and rotated them so that the solution and the air in the chamber were agitated. Under these conditions, he obtained results entirely comparable to those obtained when plates were not rotated. In the present investigation, at the lowest humidities, a further precaution was taken to make certain that the air in which the conidia germinated was actually extremely dry. Air was bubbled through three tall wash towers containing concentrated sulphuric acid before being admitted to the germination chamber.

Each germination test was based on a count of 200 conidia. In some material, it was noticed that a very small proportion of the spores had germinated before the experiment had begun. In no instance was this proportion higher than 1% and it was finally neglected in making the count of conidia germinated at the end of the test. Some shrivelled (doubtless dead) spores were nearly always found in freshly gathered material, but never more than 3% of these in a sample. The germination percentages were based on counts of all conidia in the fields examined with the microscope.

In testing the germination of the conidia in various gases, the spores on cover slips were placed in the germination chamber into which the desired gases could be admitted. As far as possible connections in this apparatus were ground glass; pure gum rubber was used only where necessary. A description of the apparatus is given by Neufeld (17).

While studying the structure of the conidiophore of *E. Polygoni*, it was found that internal details were revealed by the use of a very simple staining method. Infected leaves were folded so that the conidiophores projected beyond the leaf. The leaves were then immersed for five minutes in a 5% solution of iodine in potassium iodide and were then examined in water on a slide. The protoplast was fixed by this treatment and stained dark brown. Also, septa were especially distinct in material so treated.

## Development of the Conidiophore and Conidium of *Erysiphe Polygoni*

Because the mode of formation of the conidia has a possible bearing on the mechanism of germination, material of *E. Polygoni* on *Delphinium* was studied both when living and after being stained.

The young conidiophore is at first terete, with a blunt apex (Fig. 1*a*), and is aseptate. A slight swelling appears in the apical region and, within the cell wall, a ring of shiny material forms, becoming more and more clearly defined until it is almost as readily distinguished as the annular thickenings in angiosperm vessels (Fig. 1*b*). Considering the simple nature of the stain, it was surprising to find that excellent examples of various stages in the development of the septum were visible in every preparation.

Material is apparently added to the ring toward the inside until a disk is formed (Figs. 1*c* and 1*e*). The centre of the disk remains perforate until the conidium is mature and provides a pore which, although not discernible in

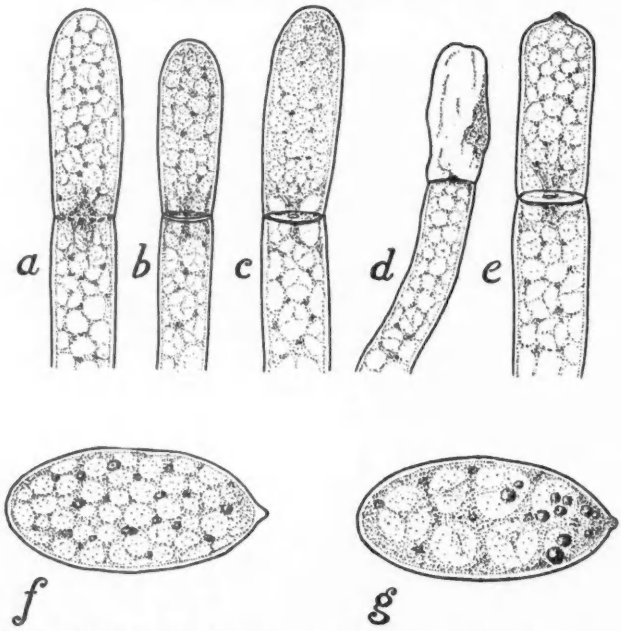


FIG. 1. *Erysiphe Polygoni: a,b,c,e, stages in the development of the perforate septum of the conidiophore; the vacuolate appearance of the cytoplasm is probably not due to the presence of true vacuoles (see text); d, conidiophore bearing a dead conidium and showing plugging of the septal pore as a result of the death of the conidium; f, conidium as seen in water showing large globules in the protoplast and smaller refractive bodies; g, same conidium after standing two hours in neutral red stain showing enlargement of globules, and heavily stained refractive bodies near papillate end.* ×*1100.*

living material, shows up splendidly when the conidiophore is immersed in iodine. Pores were also seen in septa of mycelial cells although these were not as readily found as the pores in the conidial septa.

The nature of the septum in fungi has been fully discussed by Buller (6). The pore in the septum of the conidiophore of *E. Polygoni* seems similar to what Buller has described for other fungi. To the writers' knowledge, no demonstration of the septal pore has hitherto been given for a member of the Erysiphaceae. Allen (1) stated that in *E. Polygoni* she believed the adjoining mycelial cells to be connected by cytoplasmic strands and that cytoplasmic streaming occurs in the mycelium. In a fungus such as this, the biological importance of septal pores is very great. Food material is absorbed only by the haustoria. Septal pores make possible continuity of the cytoplasm from the haustoria to the spore-bearing hyphae. Doubtless there is a constant flow of cytoplasm from the haustoria to the conidiophores. Buller (6) has demonstrated that cytoplasmic streaming occurs in many kinds of fungi and the writers endeavoured to observe it in living mildew mycelium. They were unable to do so, but this may have been because it was necessary to remove

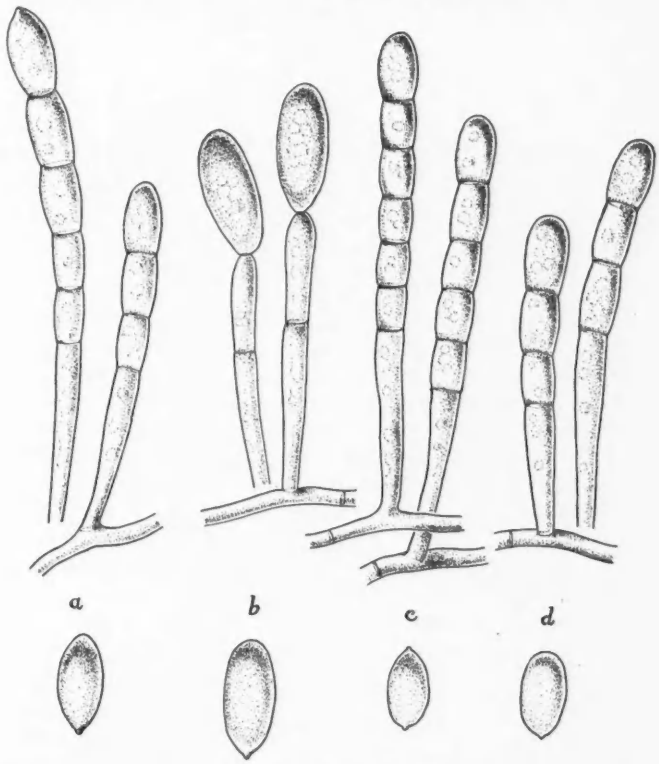


FIG. 2. *Various mildew conidia showing variation in size and shape of spores: a, Erysiphe graminis; b, Erysiphe Polygoni; c, Sphaerotheca Humuli; d, Sphaerotheca Humuli var. fuliginea.* ×500.

the mycelium from the host leaf in order to study it with the high power of the microscope. In view of the prominence of septal pores in the mycelium of *E. Polygoni*, the writers do not doubt that streaming occurs.

How the perforate septum (Fig. 1*c*) undergoes modification as the conidium matures, the writers did not determine. The end cell of the conidiophore swells and becomes the conidium, which, when mature, remains attached to the conidiophore only by a minute point of contact (Fig. 3*e*).

A detached conidium of *E. Polygoni* bears a minute shiny papilla at one end (Fig. 3*d*) and the parent conidiophore is papillate after the conidium has been dislodged (Fig. 3*g*). Such careful observers as the Tulasnes (20) did not illustrate the papilla, and other workers do not appear to have realized its importance. The present writers believe this papilla to have special significance in the germination process, as will be brought out later in this paper.

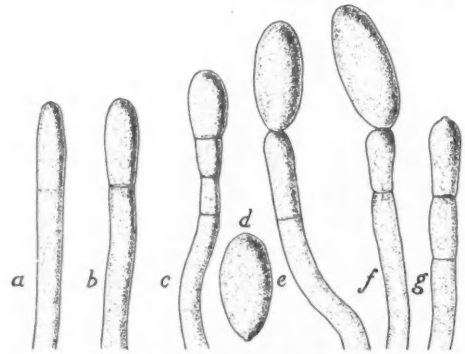


FIG. 3. *Erysiphe Polygoni: stages in the development and abstriction of the conidia.* ×500.

The conidium first formed on a young conidiophore bears only one papilla which is developed at its proximal end. Since a papilla is also formed on the conidiophore at the end attached to the conidium, the second spore formed on the same conidiophore bears two papillae, one at either end. In a mass of spore material, therefore, one finds some conidia with one papilla and some with two.

The papilla appears as a highly refractive structure, the conidium wall being thickened in the papilla region. The papilla is probably not an ordinary germ pore because the germ tube always emerges slightly to one side and not through it (Fig. 4*b*).

As stated above, Hammarlund (12) believed that the conidia of *E. Polygoni* are forcibly discharged. Yarwood denied this (22) stating that they are passively liberated. To settle this point, the writers made careful observa-

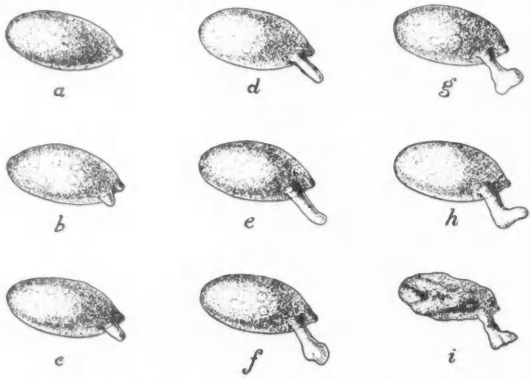


FIG. 4. *Erysiphe Polygoni: drawings, the outlines of which were made with the aid of the camera lucida, showing the germination of a conidium on a cover slip suspended over concentrated sulphuric acid. Note that there is no apparent shrinkage of the spore until death occurs (i).* ×500.

tions on living material. Young mildewed leaves of *Polygonum* were folded on slides with the undisturbed conidiophores projecting beyond the edge of the folds. The conidiophores and conidia could be readily observed with both the low- and high-power objectives of the microscope. Continuous watching for 24 hr. showed that the conidia remain attached to the conidiophores unless disturbed by shock or a breath of air. Mildewed leaves were also suspended from the top of a covered glass dish and a slide was placed underneath the leaves. After two days, during which time the dish was undisturbed, no spores had settled on the slide. It would therefore appear that, in nature, mildew conidia are dislodged by air currents, rain, or other disturbances.

Hammarlund also stated that the conidia of *E. Polygoni* are sometimes produced in chains. The writers have studied this species on numerous hosts and have found only one mature conidium attached to each conidiophore. On one occasion, mildew was allowed to develop on bean leaves in the undisturbed and saturated atmosphere of a Wardian case. A few conidiophores were found in this material bearing chains of three or four conidia. The conditions in the Wardian case, however, were doubtless highly abnormal, and it seems probable that, in nature, not more than one conidium remains attached to each conidiophore in this species.

## Structure of the Conidium

### *The Protoplast*

The conidium of *E. Polygoni* is an ovoid colourless spore measuring 36$\mu$ by 17$\mu$ on the average. The first-formed conidium on a chain has a basal papilla only, each later-formed conidium is papillate at both ends. The protoplast presents a reticulate appearance and contains numerous globules that appear like vacuoles, these being from 1/5 to 1/4 the width of the spore. At the junctures of the granular cytoplasmic strands are very small globules of a highly refractive nature (Fig. 1*f*). Nuclei were not seen, but no attempt was made to stain them.

When living conidia were immersed in a 0.2 % solution of neutral red, their appearance was markedly different from that of conidia of several other fungi in the same stain. Conidia of *Fusarium culmorum* (W. G. Smith) Sacc., *Botrytis cinerea* Pers., and *Macrophoma* sp. were immersed in neutral red, and the stain immediately entered the spores and accumulated rapidly in typical vacuoles of various sizes; these made up the bulk of the spore contents. In contrast, the mildew conidia behaved very differently. Dead shrivelled conidia would absorb the stain instantly, the entire spore contents becoming red. Living turgid conidia at first did not absorb stain at all. Only after they have been allowed to stand in the stain from two to four hours did a small amount of stain penetrate the spore walls. By the time (two or three hours) germ tubes had become visible, a considerable amount of stain had entered the spores. The part of the conidium that first showed stain was always the papilla. It became pink, and then a faint coloration appeared in that

part of the protoplast nearest the papilla. After several hours, the stain spread through the spore. However, the larger globules which have the appearance of vacuoles did not absorb neutral red. By comparison with the stain-absorbing vacuoles of the other fungi mentioned, it was concluded that the large globules in the protoplast of *Erysiphe* conidia are not true vacuoles, or that they contain some material that has no affinity for neutral red. On the other hand, the stain accumulated in considerable concentration in the small refractive globules, which may, therefore, be true vacuoles or consist of some material having an affinity for neutral red (Fig. 1g).

The writers have concluded that the spore wall of the ungerminated mildew conidium is relatively impervious to water. As germination proceeds, the permeability of the papilla probably increases since stain and water appear to enter at the papilla end and to spread slowly from there. The large "vacuole" globules are possibly not of a watery nature as they do not absorb neutral red. The stain accumulated chiefly in the small refractive bodies, from which it may be deduced that they contain water. It will be shown later that the protoplast shrinks but slightly during plasmolysis, and it would seem justifiable to conclude that the mature conidium contains very little free water prior to germination.

*The Cell Wall*

The wall of the conidium is thin (1 to 1.5$\mu$ thick) except at the papilla end, smooth, and colourless. Sudan III accumulates slightly on the outer surface of spores immersed in it, but the wall itself is not stained. It is possible that there is a layer of some waxy material on the outside of the spore wall, although it must be admitted that the spores can be "wetted" very easily. Ungerminated turgid conidia do not take up neutral red, but, if fresh spores are washed with petroleum ether, allowed to dry for a moment, and then are flooded with neutral red, the stain at once colours them heavily. The waxy covering may be dissolved by the petroleum ether, rendering the spore wall permeable to neutral red.

No perforation in the wall is discernible at the papilla end when spores are examined with the oil immersion lens, and it is thought that no perforation is present. Rather, the papilla, despite the thickness of the wall at that point, represents a permeable area in a spore wall which is elsewhere relatively impervious to water.

## Plasmolysis of the Conidia

It seemed possible that the tolerance of low humidity by the mildew conidia during their germination might in some way be correlated with a high osmotic pressure. Accordingly, an endeavour was made to determine the osmotic pressure of the mildew conidia by the plasmolytic method.

Plasmolysis in solutions of sucrose was first sought. To the writers' amazement, the spores remained unplasmolysed in the strongest concentrations of sucrose used. In fact, the conidia were able to germinate readily in a 5$M$ solution of sucrose. A few fungi such as yeasts are able to germinate and

grow in honey (14), but the failure of mildew conidia to be plasmolysed by concentrated sucrose solutions is surprising. The reason is not, as yet, clear. It was noticed that the conidia shrank considerably in strong sucrose solutions, and it may be that the shrinkage of the spore as a whole masked the shrinkage of the protoplast.

Solutions of potassium nitrate were then employed, but plasmolysis was observed only at high concentrations. Due to some irregularities in the results, detailed report upon this phase of the work will be deferred until a later date. It may be stated here, however, that the osmotic pressure of the mildew conidia is very high and probably lies between 60 and 90 atmospheres. The plasmolysis observed was normal in that the protoplast always returned to its original volume when water was substituted for the plasmolyte. That the potassium nitrate was not toxic to the conidia is shown by their ability to germinate in concentrations of that salt up to the strength inducing plasmolysis. The shrinkage of the protoplast was slight in all instances and was slow in taking place, presumably owing to the impervious nature of the conidium wall necessitating the passage of liquids into the conidium via the papilla. Even at high concentrations of the plasmolyte, the protoplast did not assume a spherical shape nor undergo marked reduction in volume. This is interpreted as additional evidence that the protoplast contains very little water that could be withdrawn during plasmolysis.

Whether or not high osmotic pressure is an aid to mildew conidia in taking up water is difficult to say. Since it will be shown in this paper that the conidia of *E. Polygoni* are capable of germinating well in a current of air dried by passage through concentrated sulphuric acid, it would appear that they are independent, in respect to germination, of their ability to take up water. Further, the question of taking up water during germination may be one of imbibition rather than osmotic pressure.

## Germination of Conidia

The conidia of *E. Polygoni* were found to germinate, on the average, in one hour and 45 min., the minimum time recorded being one hour and 15 min. The germ tube emerges slightly to one side of the end of the conidium and, in moist air, is generally straight and terete. In dry air, it may be short and often convoluted (Fig. 5*c*). Only one germ tube was found to be produced by each spore.

The writers have heard the criticism made of Yarwood's work that the germ tubes observed by him (produced under conditions of low humidity) might be abnormal swellings on the spores. This idea is entirely erroneous, for the writers have observed the development of thousands of normal germ tubes on spores kept in extremely dry air.

Conidia of various mildew species were subjected to germination tests under conditions of humidity ranging from zero (approximately) to 100%, according to the procedure outlined above. The temperature during these experiments

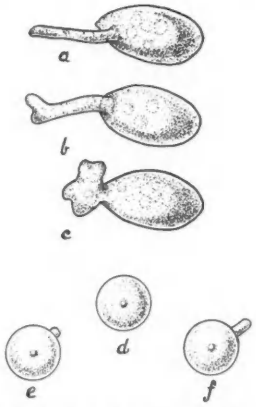


FIG. 5. *Erysiphe Polygoni: a, b, c, variation in form of germ tube at different humidities, a and b in moist air, c, in very dry air; d, e, f, drawings made with the aid of the camera lucida showing a conidium germinating on a cover slip suspended over concentrated sulphuric acid and demonstrating that the conidium does not change in outline during germination when seen from above.* ×500.

varied but slightly from 22° C. although no attempt was made to control it. The results of these experiments are given in Table I.

The results fully uphold Yarwood's contentions, for it will be seen from Table I that (except for the two varieties of *S. Humuli*) the mildews examined germinated as well at low humidity as at high. In no experiment was there a

TABLE I

PERCENTAGE GERMINATION OF POWDERY MILDEW CONIDIA UNDER DIFFERENT CONDITIONS OF HUMIDITY AT 22° C.

| Organism | Relative humidity in germination chamber, %[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 2.5 | 4.5 | 7.5 | 10.5 | 20 | 32.5 | 54.7 | 79.5 | 90 | 95 | 100 |
| *Erysiphe Polygoni* from *Delphinium* | 36<br>21<br>23 | 46<br>16<br>23 | 20<br>—<br>— | 24<br>18<br>21 | 27<br>28<br>39 | 21<br>18<br>39 | 31<br>37<br>38 | 24<br>34<br>31 | 41<br>26<br>32 | 43<br>29<br>36 | 52<br>27<br>29 | 12<br>31<br>29 |
| *Erysiphe Polygoni* from *Polygonum* | 33 | 6 | 1 | 4 | 28 | 16 | 2 | — | 2 | 4 | 0 | 7 |
| *Sphaerotheca Humuli* from rose | 0 | 0 | 0 | 0 | 0 | 0 | 4 | — | 0? | 11 | 6 | 14 |
| *Sphaerotheca Humuli* var. *fuliginea* from pansy | 0 | 0 | 0 | 0 | 0 | 0 | 6 | — | 0? | 22 | 14 | 24 |
| *Erysiphe graminis* from *Poa pratensis* | 22 | 16 | 14 | 11 | 15 | 31 | 11 | — | 20 | 13 | 12 | 10 |

[1] *Germination percentages given only as nearest whole numbers.*

clear-cut optimum humidity, and the writers therefore agree with Yarwood in concluding that mildew conidia are tolerant of a wide range of humidity as regards germination or, to put it another way, are independent of humidity.

Neither of the two varieties of *S. Humuli* tolerates conditions of very low humidity, no germination being observed below 32.5% and but little below 90%. However, as compared with the requirements of most fungi, 32.5% is remarkably low. Neither the rose nor the pansy mildew is generally found in hot dry locations, and it is not surprising that they should be less tolerant of low humidity than the other kinds tested, all of which may be found in open, dry situations.

In the above experiments, a possible source of error was considered. It was thought that some time might elapse before conditions of low humidity were really established in the germination chambers. Thus, if the laboratory air in the summer had a relative humidity of, say, 50% at the time of the test, the conidia placed in the closed chamber would begin their germination at that humidity and might be subjected to low humidity only after the regulating solution had withdrawn moisture from the air of the germination chamber. And since the conidia germinate in a very short time, this lag in the establishment of low humidity might lead to serious error. In order to leave no possibility of doubt regarding the ability of the conidia to germinate in extremely dry air, the following experiment was carried out.

Freshly gathered conidia on cover slips were placed in a glass bottle provided with a ground glass stopper and two tube outlets. One of the outlets was connected to a series of three tall wash bottles and these were filled with concentrated sulphuric acid. Air was drawn through the whole apparatus immediately after the spores were placed in the germination chamber. The air, bubbling very slowly through the wash bottles, must have been very desiccated when it reached the conidia. The air originally present in the germination chamber was, in this way, removed at once, and one could be certain that the conidia had been subjected to dry air from the beginning of the test. Four such tests were made for which the average germination percentage was 35.5%. The average for spores allowed to germinate in the laboratory air at the same time was 52%. This experiment indicates that there is no doubt about the ability of mildew conidia to germinate in dried air.

## Constancy of Volume of Conidia during Germination

Yarwood (23) stated that he had observed a shrinkage of as much as 24% in volume of turgid conidia when these were germinating in an atmosphere of 80% relative humidity. The writers made a special effort to obtain confirmation of this finding, with negative results. It had been found that excellent germination may be obtained when the conidia are merely caused to fall on dry slides and allowed to germinate in the air of the laboratory. Conidia of *E. Polygoni* from *Delphinium* and from *Polygonum aviculare* were dusted on dry slides and then placed on the stage of the microscope. Camera lucida drawings were made of several conidia under the high power at inter-

vals of about 20 min. (Fig. 4). In another series of observations, measurements were taken at intervals with an ocular micrometer. During the experiments, which were made on different days, the air of the laboratory ranged from 65 to 80% relative humidity.

Up to three hours, within which time the conidia had germinated, the writers failed to observe any change in volume of the dozens of conidia observed continuously. It was suggested to the writers by Professor A. H. R. Buller of the University of Manitoba, that depression of the conidia along the short axis might take place without resulting in change of outline seen from above the spore, when the spore lay with its long axis parallel to the glass slide. However, a few spores were found that had landed on one end, and several of these were watched carefully during germination. Here too, no change in outline could be observed (Fig. 5–*d*, *e*, *f*). Tests were also made at zero relative humidity with a similar result.

The writers are forced to conclude that, in their material at least, there was no change in volume of the spores during germination. Yarwood's measurements, from which he deduced that shrinkage takes place, were made after 5, 10, and 24 hr. The writers have rarely found that germinated conidia remain alive on slides for more than five hours. After about four hours, growth ceases (Fig. 4*h*), and one observes a marked change in the appearance of the protoplast which becomes darker and denser. At about this time a marked wrinkling of the spore is evident and the whole conidium shrinks (Fig. 4*i*). It seems certain that the conidium is dead, however, before the shrinkage occurs.

## Non-Germination of Mildew Spores *in situ*

Having established the fact that the spores of some powdery mildews are independent of moisture for germination, the question naturally arises: are the conidia capable of germinating *in situ*? In nature, mildew conidia produced on the conidiophores above the surface of the leaf might be expected to germinate as soon as they are produced.

Examination of the illustrations of such careful observers as the brothers Tulasne (20) fails to reveal any spores germinating while still attached to the conidiophores.

Several times the writers kept material of *E. Polygoni* under constant observation for a period of 24 hr. The mildewed leaves were folded so that the conidiophores projected into the air and could be readily examined under low power. As a result of these observations, it may be stated that the conidia do not germinate as long as they remain attached to the conidiophores. But one has only to shake the conidia on to a dry slide to see them germinate, and it is necessary to offer some explanation as to why they remain ungerminated while attached to the conidiophores.

At first it was believed that, as long as the conidia remain attached, some chemical substance on or in the conidiophore might inhibit germination. To

test this hypothesis, a large quantity of mildew mycelium was carefully scraped from infected leaves and added to a small quantity of sterile water, and thoroughly macerated. Fresh conidia were then suspended in the water and macerated mycelium. As control experiments, conidia were suspended in sterile distilled water and in water containing macerated host tissue. Mildew conidia do not germinate well in water, but 15% germination was obtained in the sterile water and in host tissue. The same amount of germination was obtained in the macerated mycelium, indicating strongly that there is no chemical substance present in the mycelium to inhibit the germination of the conidia.

Next, the matter of maturity was considered. It has been shown that the conidia become disjoined from the conidiophores by shock or wind and it seemed possible that the conidia might be immature until just before they are able to be dislodged. If this were so, conidia artificially detached prior to maturity could not germinate when detached.

A mildewed leaf was held firmly with forceps and then shaken violently in order to dislodge all conidia possible. The leaf was then examined under the low power of the microscope and several conidiophores bearing firmly attached conidia were found. By means of careful micromanipulation, these immature conidia were removed. They were dislodged only after considerable probing with the needles of the manipulator. Despite the fact that not 1 of the 10 conidia so obtained was sufficiently mature to drop from its conidiophore, 9 of the 10 germinated after being detached. It would appear from this observation that, provided the formation of the conidium is complete, it can germinate, whether ready to drop off of its own accord or whether artificially dislodged. The suggestion that the conidia do not germinate *in situ* because they are undergoing a process of maturation is not borne out by this observation.

## Relation of Carbon Dioxide to Germination

In considering the conditions within the mature conidium that might be responsible for its failure to germinate as soon as it is produced, it may be well here to restate certain facts regarding its structure. The septum which abstricts the terminal conidium is, at first, perforate, providing for cytoplasmic connection with the conidiophore and mycelium below (Fig. 1*c*). Later, the septum becomes entirely closed and the conidium may be supposed to be separated physiologically from the conidiophore. After separation, the continued respiration within the conidium might do two things: (1) increase the internal concentration of carbon dioxide and, at the same time, (2) decrease the internal concentration of oxygen. It has already been shown in this paper that the conidium wall is relatively impervious to water except at the papillate end. If it be assumed that it is also relatively impervious to gases, then, as long as the conidium remains attached to the conidiophore, either the accumulation of carbon dioxide within the mature conidium or the low concentration of oxygen might prevent its germination. The conidium appears

so firmly attached to the conidiophore that it seems safe to assume that the permeable papilla would not be exposed to the outside air until after the conidium had become detached.

To test this theory, mildew conidia were first subjected to an atmosphere of air containing excess of carbon dioxide. Freshly dislodged conidia collected on glass cover slips, were placed in a large glass-stoppered wash bottle provided with two exit tubes. Air was drawn into the chamber and a small proportion of carbon dioxide (approximately 1 part in 10) was allowed to mix with the air entering the germination chamber. The gas mixture was allowed to pass slowly through the germination chamber for 30 min. Ground-glass cocks on either side of the germination chamber were then closed and the spores allowed to remain in the gas mixture. A control experiment was set up using a duplicate apparatus lacking the arrangement for adding carbon dioxide.

After two hours, excellent germination had taken place in the control apparatus, while in the presence of added carbon dioxide no spores had germinated. The ungerminated spores were then removed from the germination chamber in which there was added carbon dioxide and they were allowed to stand exposed on the laboratory table. Two hours later, these spores were found to have germinated. The results of the experiment are presented in the following table.

TABLE II

EFFECT OF CARBON DIOXIDE ON GERMINATION OF MILDEW CONIDIA

| Trial No. | Percentage germination | | |
|---|---|---|---|
| | In chamber with added $CO_2$ | In chamber with air | Same conidia shown in Column 2, two hours after removal from $CO_2$ chamber |
| 1 | 0 | 45 | 43 |
| 2 | 0 | 50 | 55 |
| 3 | 0 | 50 | 44 |

From these results it may be concluded: (1) that carbon dioxide in concentration of approximately 10% by volume prevents mildew conidia from germinating during the two hours required for germination of conidia exposed to atmospheric air; and (2) that conidia that have been held in carbon dioxide for two hours germinate as well after they have been removed from the carbon dioxide as though they had not been subjected to experiment.

The writers hoped to be able to extend the above experiment to find the critical concentration above which no germination could occur. However, much difficulty was experienced at first in obtaining consistent results in the experiment. This proved to be due to the extreme toxicity to the spores of vulcanized rubber used in the form of rubber tubing and stoppers in the apparatus. The source of trouble was not located until late in the work

and it was not possible to put the experiment on a more quantitative basis. However, the results are so clear-cut as to seem convincing, even in their unrefined form.

## Relation of Oxygen to Germination

Experiments were carried out to determine whether or not the conidia of *E. Polygoni* are capable of germinating in an atmosphere in which the proportion of oxygen is less than that present in ordinary air. At first an attempt was made to remove oxygen from atmospheric air by passing the latter very slowly through three wash bottles containing strong alkaline pyrogallol. The conidia were placed in the germinating chamber as in the previous experiments, and a control apparatus was set up in which washed air passed directly over the conidia without first passing through pyrogallol. The results of several tests were entirely negative; about 65% germination was obtained in the air which had passed through pyrogallol as well as in the control apparatus.

The conclusion to be drawn is either that the conidia are capable of germinating in oxygen-free air or that the pyrogallol did not remove sufficient oxygen to cause any checking of germination. The latter explanation seemed the more probable.

A further experiment was conducted in which a heated combustion tube was connected between the pyrogallol wash bottles and the germination chamber. Into the combustion tube was inserted a roll of freshly reduced copper gauze which was heated as the air from the intake was drawn slowly over it. Again negative results were obtained.

Conidia were then placed in an atmosphere of "tank" nitrogen. The gas was allowed to pass through the apparatus for 20 min. only, to sweep out the air, and pinch cocks were then closed on either side of the germination chamber. In the control experiment the conidia were subjected to ordinary air in a duplicate apparatus. This time, positive results were obtained as shown in Table III.

TABLE III

EFFECT OF NITROGEN ON THE GERMINATION OF MILDEW CONIDIA

| Trial No. | Percentage germination | | |
|---|---|---|---|
| | In chamber with nitrogen | In control chamber | Same conidia shown in Column 2, two hours after removal from nitrogen chamber |
| 1 | 0 | 12 | 10 |
| 2 | 2 | 80 | 80 |
| 3 | 2 | 75 | 75 |
| 4 | 0 | 27 | 18 |
| 5 | 6 | 80 | 80 |
| 6 | 0 | 27 | 30 |
| 7 | 3 | 80 | 80 |

It will be seen that in all trials, germination was prevented or markedly checked when the conidia were held in nitrogen. In trials Nos. 1, 4, and 6, no germination had occurred in two hours in nitrogen, whereas, in the controls, 12, 27, and 27% germination, respectively, was observed. In all trials, the germination of conidia held for two hours in nitrogen was approximately the same after the conidia had been removed from nitrogen and allowed to germinate on the laboratory table as though they had not been subjected to experiment.

The small percentages of germination in nitrogen observed in trials Nos. 2, 3, 5, and 7, may be explained by the fact that "tank" nitrogen contains as high as 4% oxygen and this, presumably, is sufficient to allow a small proportion of spores to germinate.

From these experiments it may be deduced (1) that lack of oxygen or a very low concentration of oxygen prevents germination of the mildew conidia and (2) that, at least for a period of two hours, conidia are not permanently injured by lack of oxygen as shown by the fact that they germinate well when oxygen is provided.

To date, the writers have not determined the critical concentration of oxygen below which no germination can take place. This would have to be carried out using chemically pure nitrogen to which could be added as much oxygen as desired.

### Toxicity of Vulcanized Rubber to Mildew Conidia

During the course of the above experiments, considerable difficulty was experienced at first in obtaining any germination within the apparatus used for examining the effect of gases on germination. Finally it was found that both ordinary rubber tubing and rubber stoppers are extremely toxic to mildew conidia. When ground-glass connections and pure gum-rubber tubing were used in the apparatus, excellent germination within the apparatus was obtained. The high degree of toxicity of vulcanized rubber seemed to warrant some actual tests, and the following experiments were therefore performed.

Spores were suspended on dry cover slips over Van Tieghem cells. In each cell was placed a small fragment of rubber stopper (about 2 mm. in diameter). Twenty-four cells were arranged along with a series of the same number of controls not containing rubber fragments and a series of cells containing fragments of pure gum rubber. The results of this test are given in Table IV.

This experiment shows that some substance in vulcanized rubber is highly toxic to the spores of *E. Polygoni*. Since non-vulcanized rubber is not toxic and vulcanized rubber contains sulphur, and also since it is a matter of common knowledge that sulphur is a good fungicide for the control of diseases caused by powdery mildews, it would appear probable that the substance present in rubber that is toxic to the mildew spores is a compound of sulphur. The conidia failed to germinate when they had been removed from the cham-

TABLE IV

EFFECT OF VULCANIZED RUBBER ON GERMINATION OF MILDEW CONIDIA

| Percentage germination after two hours[1] | | |
|---|---|---|
| In precence of vulcanized rubber | In presence of non-vulcanized rubber | In control |
| 3.5 | 37 | 41<br>57 |

[1] *Average of 24 counts of 100 conidia each.*

bers containing vulcanized rubber, which shows that they had been injured permanently. A search for the toxic principle of vulcanized rubber might lead to the discovery of an excellent means of control of powdery mildews[1].

## Discussion

Unlike the spores of all other fungi, so far as is known, the conidia of certain of the Erysiphaceae are capable of germinating under conditions of extremely low humidity. The writers agree with Yarwood (23) in concluding, not that low humidity is essential to the luxuriant development of mildew, but rather that, unlike other fungi, the powdery mildews are capable of developing over a wide range of relative humidity and that they are well adapted to dry atmospheric conditions. This adaptation appears to be related to the physiological and morphological peculiarities of the conidia.

The conidium, as it matures, becomes walled off from the conidiophore by a septum but remains firmly attached to the conidiophore, contact being maintained between a papilla at the proximal end of the conidium and a papilla at the distal end of the conidiophore. The wall of the mature conidium appears to be relatively impervious to water and probably to gases except at the papillate end through which stain has been shown to enter.

After maturation, the conidium remains on the conidiophore unless detached by shock, wind, etc., and in this position it is sealed, if not hermetically, at least sufficiently to prevent the ingress of oxygen from the outside or release of carbon dioxide from the inside to the extent necessary to initiate the germination process. Thus it fails to germinate *in situ*, even though the relative humidity of the air surrounding the spore may be suitable for germination.

Upon being dislodged from the conidiophore, the seal formed by the firm contact of the papilla with the conidiophore is broken. The end of the papilla is then exposed to the surrounding air and, being more permeable than the remainder of the conidium wall, it allows carbon dioxide to pass out from the protoplast and oxygen to pass in.

[1] *Since these statements were written, the senior author has carried out experiments which show that as little as 0.01 gm. of vulcanized rubber per 100 cc. of air will prevent mildew spores from germinating on dry slides in Petri plates.*

The writers have not been able to prove, up to the present, whether low oxygen concentration or high carbon dioxide concentration is responsible for checking germination, but they are inclined to the belief that it is the accumulation of carbon dioxide within the protoplast that is of greater importance in this regard.

When Yarwood's work was first published, the criticism was raised (but apparently not published) that the increase in volume that would seem bound to result from the spore producing a germ tube, must come from the uptake of water, and the problem arose as to how the spore could withdraw water from dry air. It is suggested by the writers' preliminary experiments that the conidium of *E. Polygoni* has a very high osmotic pressure, which might account for the ability of the conidium to withdraw water from moderately dry air. This mechanism does not seem, however, to account for the germination of spores in a current of air dried by passage through sulphuric acid.

The protoplast of the conidium appears quite unlike that of the spores of *Fusarium culmorum*, *Botrytis cinerea*, and *Macrophoma* sp. with which it has been compared. In those fungi there are large watery vacuoles which readily absorb neutral red, while no such vacuoles appear in the ungerminated conidium of *E. Polygoni*. There is probably very little water present in the mildew conidium, the protoplast consisting of a gel-like material.

Upon the release of carbon dioxide from the conidium, respiration would begin if sufficient oxygen were present. Conceivably, as a result, the viscid protoplast would be converted into materials more labile and voluminous, and the increase in volume necessary for the production of a germ tube might come from this source alone.

It has been stated that neutral red enters living conidia, at first extremely slowly, and then more rapidly as germination proceeds. Probably the sporelings do require water after a time; and, under natural conditions, they might be in a position to absorb it. At low humidity, on dry slides, the germ tubes seldom become as long as the spores and they soon die. This may well be due to the lack of water necessary for further development.

Quite regularly, many conidia shrivel early in germination tests at low humidity. One may ask: why, under dry conditions, do some conidia germinate while others shrivel and die? One explanation that might be offered is that, for some reason, in the conidia that shrivel, the papilla does not function normally. It has been noted that dead conidia absorb neutral red immediately upon immersion in it, which would indicate that the papilla end of the spore becomes entirely permeable when death of the spore takes place. Possibly, in some spores, the papilla is too permeable and, under dry conditions, it may allow the contents of the spore to suffer through drying. The writers hope to throw more light on this and other problems by means of further research now in progress.

Finally, the writers suggest that, at this stage of development of the present problem, caution should be observed in applying the ideas brought forth in this paper to questions of the epidemiology of mildew on crop plants.

There seems no doubt that certain mildew species tolerate extremely low humidity. However, infection (involving the establishment of the mycelium on the host and haustoria within the host cells) may well reach its optimum under conditions different from those which bring about optimum germination. The writers' experience with *E. Polygoni* leads them to believe that higher humidity may be necessary for infection than is necessary for germination. Further research along this line is needed to clarify the situation.

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The writers are indebted to Professor A. H. R. Buller, Professor Emeritus of the Department of Botany of the University of Manitoba, who, from time to time, critically discussed the work with the senior author and made valuable suggestions regarding the preparation of the manuscript. Professor William Leach, Professor of Botany at the University of Manitoba, also gave the writers the benefit of his criticism. It is a pleasure to acknowledge the invaluable aid given by these gentlemen.

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# Canadian Journal of Research

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## THE EFFECT OF TEMPERATURE ON THE CRITICAL OXYGEN PRESSURE FOR HEART-BEAT FREQUENCY IN EMBRYOS OF ATLANTIC SALMON AND SPECKLED TROUT[1]


BY KENNETH C. FISHER[2]



### Abstract

Intact embryos of speckled trout and Atlantic salmon were exposed to solutions of oxygen and nitrogen in distilled water. It was observed that as the partial pressure of oxygen was reduced a pressure was found below which the frequency of the heart-beat was not maintained at the normal level characteristic of higher partial pressures of oxygen. A "critical" partial pressure of oxygen for heart-beat frequency can therefore be said to exist. Data have been obtained from which its value at five different temperatures can be determined. The critical pressures for the two organisms are similar, rising from approximately 3 to 5 mm. of mercury at 1.5° C. to 40 to 50 mm. of mercury at 20° C. Possible mechanisms leading to the establishment of a critical oxygen partial pressure are discussed. It seems likely that diffusion is not the limiting factor in these preparations so that the critical pressure, and its temperature coefficient, must be the property of the intracellular respiratory systems concerned. The finding that the logarithm of the critical partial pressure can be represented as a linear function of the reciprocal of the absolute temperature is consistent with this view. Temperature exercises a more pronounced effect on the critical oxygen pressure of the pacemaking process, than it does on the over-all velocity of that process as indicated by the normal frequency of the heart. It is concluded that the critical pressure is a characteristic of the chemical systems in the pacemaking cells of the heart.


### Introduction

The oxidation mechanism in the pacemaking cells of the heart has recently been investigated in intact fish embryos by the use of carbon monoxide (2). An irregularity occurred in those experiments which was probably due to the low partial pressure of oxygen employed, rather than to the poisoning of some enzyme by carbon monoxide. It was thus necessary, before proceeding further with such experiments, to establish the partial pressure at which effects due only to oxygen lack might be expected to appear (i.e., to determine the *critical* pressure). In addition, since both Tang (10) and Kempner (5) have indicated that the critical oxygen partial pressure probably has a high temperature coefficient, it was desirable that the determinations be made at


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a series of temperatures. It is apparent that the temperature coefficient of the critical partial pressure of oxygen for the heart-beat frequency may itself serve to further characterize the chemical systems of the pacemaking process. Moreover, a knowledge of the critical pressure in fish embryos and its variation with temperature is important in ecological and cultural problems. Data concerning the critical partial pressure for the heart-beat frequency at several different temperatures in embryos of salmon and trout are reported in this paper.

## Materials and Methods

Embryos of Atlantic salmon, *Salmo salar* Linnæus, and speckled trout, *Salvelinus fontinalis* Mitchill, were maintained in the laboratory in the manner described by Armstrong and Fisher (1). The organisms were used from a period approximately one month before they would naturally have hatched, until the increased pigmentation made observation of the heart difficult. If hatching had not yet occurred spontaneously, the chorion was removed a day or more before the organism was used for an experiment.

In each experiment the organisms were initially exposed to aerated distilled water. For subjection to various partial pressures of oxygen four embryos were held in a glass tube through which was maintained a flow of water (approximately 10 ml. per min.) containing oxygen at the desired partial pressure (2). Four such tubes were held in a rack immersed in a constant temperature water-bath so that the effects of four different partial pressures of oxygen were usually determined simultaneously. The results will be given as the average heart-beat frequency of the fish in each tube.

The constant temperature water-bath was cooled below room temperature by the continuous addition of cold water or by means of an electric refrigerating unit. Electric heaters controlled by a thermoregulator then raised the bath to the desired temperature. The sensitive element in the control circuit was a nickel resistance thermometer assembled for the purpose in this laboratory. The unbalance of the Wheatstone bridge containing the thermometer was used to operate a galvanometer relay which in turn adjusted the heating (cf. 7). Such a system is extremely convenient for rapidly and accurately shifting the temperature to predetermined values. With six volts across the bridge, temperatures were constant to $\pm 0.05°$ C.

Gas solutions containing oxygen at partial pressures from 150 mm. of mercury to approximately 10 mm. were prepared by saturating gas-free distilled water with oxygen–nitrogen mixtures. To obtain a continuous supply of water containing oxygen at pressures below 10 mm. of mercury, water was siphoned out of a flask that was kept at a constant level, and that was electrically heated at a constant rate (Fig. 1). The oxygen concentration in the heated water was made high or low by varying the rate of inflow and outflow. Since the solutions were rendered toxic by passage through tin, lead, copper, or aluminum tubing it was necessary to use the more fragile glass tubing throughout for cooling coils, etc. Rubber, though used at certain

joints where flexibility was required, was kept to a minimum since at the lower partial pressures appreciable quantities of oxygen pass through the wall of ordinary rubber tubing from the atmosphere. In some experiments this permeability of rubber to oxygen was employed with advantage to secure low pressures of oxygen.

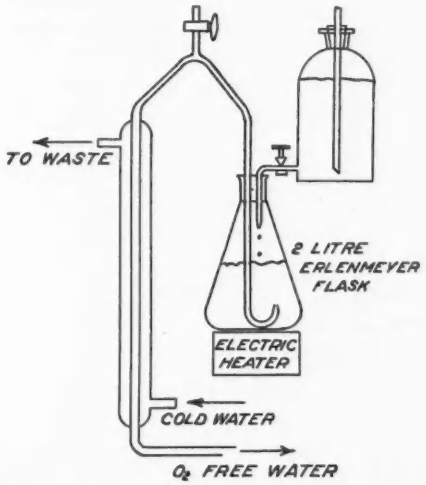


FIG. 1. *Apparatus which produces continuously water with a low oxygen content.*

After passage through a tube of embryos, the irrigating solution was led by means of a capillary to the bottom of a 10 ml. specific gravity bottle. The overflow passed to waste as drops, the frequency of which was a measure of the rate of flow past the embryos. As desired, the specific gravity bottles, filled to overflowing, were removed for analysis of the water for oxygen and a new bottle was immediately placed in position. On the average between four and five analyses were made on the gas solution passing over each set of four organisms, the samples being taken at intervals throughout the experiment. The variability of the partial pressure during an experiment was within the limits of reproducibility of duplicate analyses. In discussing the results therefore, the average of the analyses will be reported.

As stated by Lund (6) it was found also in this work that the Winkler method for the determination of dissolved oxygen can be adapted for use on a 10 ml. sample by employing smaller quantities of the reagents than are used in the standard procedure. Analyses were performed by the Winkler method as described by Sutton (9) but modified for water samples of 10 ml. by using only 0.05 ml. of the manganous chloride, 0.15 ml. of the alkaline iodide, and 0.2 ml. of the concentrated hydrochloric acid. The quantities of oxygen actually determined in this research varied for the most part between $2 \times 10^{-5}$ and $2 \times 10^{-6}$ gm. In this range the average difference between duplicate

analyses appeared to be an absolute rather than a relative quantity. As the method was employed it corresponded to approximately $\pm$ 4 $\times$ $10^{-7}$ gm.

The partial pressures were calculated from the analytically measured quantities ($M$) of dissolved oxygen by the formula

$$p.p.O_2 = 760 \frac{M}{q}$$

$q$ being the amount of oxygen dissolved in water at the temperature of the experiment when its partial pressure is 760 mm. of mercury (values of $q$ were obtained from data given in the Handbook of Chemistry and Physics, 19th ed., 1935, Chemical Rubber Publishing Co.).

## Results

During the preliminary period of exposure to aerated distilled water, the frequency of the heart-beats often showed a tendency to drift slightly but after the first hour or two became quite constant (the average deviation of the frequency from the average being approximately 2%). This constant frequency will be referred to as the normal frequency.

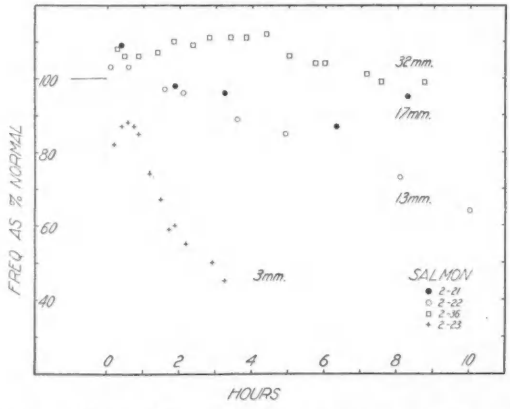


FIG. 2. *The effect of low partial pressures of oxygen on the frequency of the heart-beats in intact embryos of Salmo salar. At zero time the oxygen pressure was changed from approximately 150 mm. of mercury to the values indicated. The heart-beat frequency is expressed as a percentage of the normal frequency and each point is the average of observations made on four organisms at 6° C.*

Fig. 2 illustrates typical observations recorded upon subjecting salmon embryos to water containing oxygen at partial pressures below that in air. At 17 and 32 mm. the frequency did not depart consistently from the normal. At the other two oxygen concentrations shown, it fell with time. Apparently when supplied at partial pressures of 3 or 13 mm. oxygen cannot be utilized at the normal rate by the pacemaking cells, so that the frequency of the heart-beat falls. There must exist a pressure in the region of 13 to 17 mm. that is just sufficient to support the normal rate of oxygen consumption and

consequently the normal frequency. Such a pressure is usually designated the *critical* oxygen partial pressure. One may therefore speak of the lowest concentration of oxygen that will maintain the normal rate of the heart, as the critical partial pressure for heart-beat frequency.

As in the experiments described by Haywood *et al.* (4), the frequency fell more rapidly the lower the oxygen pressure, and the effects were completely reversible unless the oxygen lack was greatly prolonged. It will be noted that there is little, if any, indication that the frequency falls to an asymptotic level such as was seen when these preparations were exposed to oxidative poisons (1, 2, 3). Various degrees of auriculoventricular block developed as the frequency fell, and finally irregularities occurred even in the auricular beat. When this stage was reached the experiment was usually terminated (or the ability to recover was tested), as beyond this point the frequency observed probably does not represent accurately the activity of the pacemaking cells.

It is notable that the frequency does not fall instantaneously from its normal value to zero upon establishing conditions of oxygen lack, but that instead the change is gradual. It is quite inconceivable that it should require more than a few minutes to establish an equilibrium between the oxygen concentration to which the pacemaking cells are exposed, and one newly set up outside the organism. The comparatively slow rate of change of the frequency must, then, be a property of the pacemaking system after the partial pressure of oxygen has been brought to a new level. This inertia towards change suggests that the mechanism has, in effect, a "reserve of frequency". It is as if the heart rate is proportional to the concentration of a susbtance that is gradually utilized following the establishment of an oxygen concentration below that necessary for normal function. As would be expected, and as Fig. 3 indicates, this reserve is depleted more rapidly, the higher the temperature.

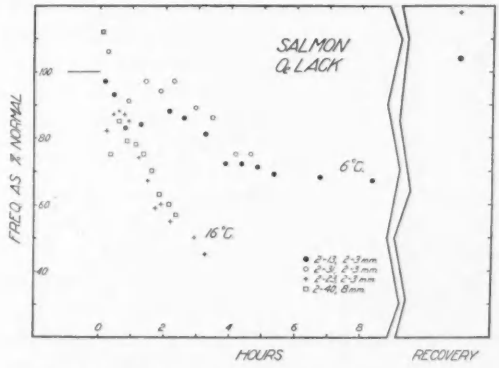


FIG. 3. *The effect of temperature on the rate at which the heart-beat frequency changes upon subjection of the intact embryo to low oxygen partial pressures.*

It is apparent from the data already given, that by subjecting groups of organisms to different partial pressures of oxygen, pressures can be distinguished that have no effect on the frequency, while others are clearly too low for the maintenance of the normal frequency. The results of a series of such observations are combined in Fig. 4. The ordinate of each point indicates

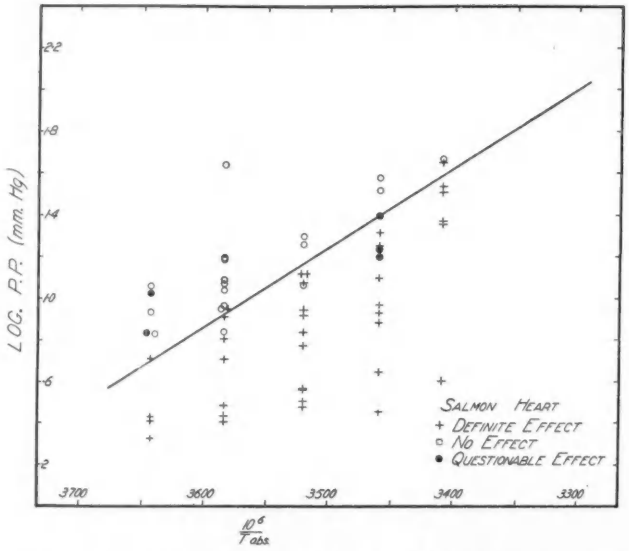


FIG. 4. *A composite plot of data obtained for the heart of the salmon embryo illustrating the critical oxygen partial pressure and its variation with temperature. See text for details.*

the logarithm of the partial pressure of oxygen throughout that experiment while the abscissa represents the reciprocal of the absolute temperature at which it was performed. Those experiments in which the average frequency of the heart-beats in the four embryos employed fell unmistakably as a result of the lowered oxygen concentration, are designated by crosses, those in which no effect was detectable, by circles. Borderline cases in which the effect was so slight as to be within the possible variation of a control are indicated by solid circles. The trend of the points is such that practically all of the crosses are below the line drawn through the data (in that region the oxygen concentration was below the critical value) while most of the circles lie above it; there, the experiments were preponderantly above the critical oxygen partial pressure. The disposition of the points is such that this line can be considered a fairly accurate representation of the variation of the critical partial pressure with temperature. The logarithm of the critical pressure can therefore be expressed as a linear function of the reciprocal of the absolute temperature. The data in Fig. 4 were obtained on embryos of Atlantic salmon. In Fig. 5 the equivalent data for embryos of speckled

trout are given. The critical pressures for these two species and the mode of its variation with temperature are grossly similar.

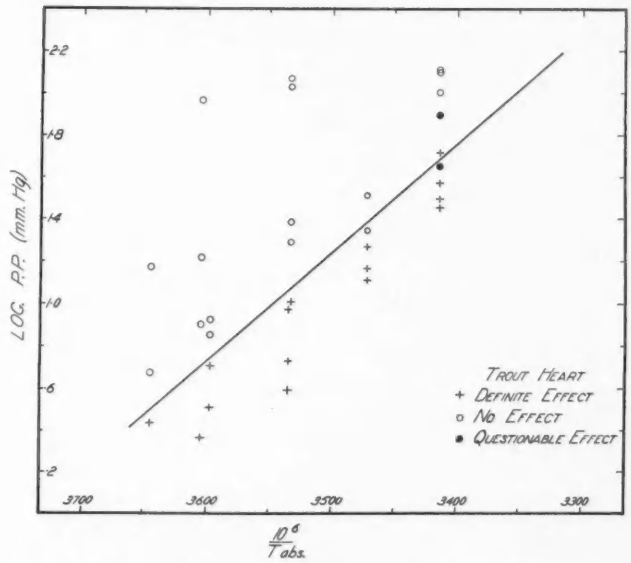


FIG. 5. *The data obtained for speckled trout treated as salmon (Fig. 4).*

The slopes of the lines in Figs. 4 and 5 are measures of the effect of temperature on the critical oxygen partial pressure. To evaluate the slopes the lines may be described by equations having the form

$$4.6 \log [O_2] = \frac{H}{T} + C \qquad (1)$$

where $[O_2]$ is the critical partial pressure of oxygen at the absolute temperature $T$, $H$ is a constant evaluating the slope of the line and $C$ is also a constant. $H$ alone is significant in the present connection.

Some difference of opinion is possible with regard to the slope of the lines drawn in the two figures. For salmon, eight individuals who examined the data independently placed lines for which the value of $H$ varied from 16,200 to 18,700. From the line shown $H=17,700$. Similarly, as judged by the same individuals, $H$ for the data of Fig. 5 lies between 21,300 and 25,800, being 23,900 for the line drawn. It seems therefore that the estimation of the value of $H$ from Figs. 4 and 5 may vary by $\pm 5\%$ (this being the average deviation of the value determined by each of the individuals from the average of the eight values).

It will be appreciated that the effect of temperature on the rate at which the frequency falls at low partial pressures of oxygen, complicates the decision as to whether or not a given pressure is above or below the critical value. If

the census to determine the effect of the low pressures is taken after a given duration of exposure, the critical pressure at low temperatures may appear to be relatively higher than the true value. This situation could result from the possibility that a partial pressure determined to be above the critical value at a low temperature, might have proved to be below it if a greater duration had been allowed. An artifact such as this will cause the critical values reported for the lower temperatures to be higher than the true values, and the effect of temperature will therefore appear less than it actually is. In the experiments on salmon, the average length of the exposure to reduced pressures was eight and one-quarter hours. The data given in Fig. 4 were obtained by examining the observations at the conclusion of each experiment. The results are not significantly different if considered after an exposure of only five and one-half hours. However, two hours after establishment of the lower pressure, the observations suggest a much smaller temperature effect. It is clear that the values recorded in the figure are free of any discrepancy that might be due to the slower changes of frequency at the low temperatures.

The data for trout are less complete, the average exposure to the low concentration of oxygen being only four and one-half hours. There is a definite possibility in this case that the indicated effect of temperature is smaller than the true value. $H$ as determined for salmon and trout may be more different therefore than at present appears.

It will be suggested in the discussion that $H$ is a quantitative characteristic of one step in the sequence of chemical reactions that constitute the pacemaking process (cf. 2). That step is the combination of oxygen with a respiratory catalyst, $H$ measuring the effect of temperature on the equilibrium constant of the reaction.

The effect of temperature on the over-all velocity of the pacemaking system can likewise be estimated from the data obtained in this research, for the normal rate of the hearts is a measure of that velocity. It follows that from a consideration of the normal frequencies at the different temperatures, an indication is obtained of the effect of temperature on the velocity of the pacemaker. While $H$, and the equivalent constant $(=\mu)$ descriptive of the temperature effect on the normal frequencies are probably not related in a simple fashion, it is of interest to compare them. In Fig. 6 the logarithms of the normal frequencies have been plotted against the reciprocal of the absolute temperature at which they were observed. Every point is the arithmetical average of the heart-beat frequencies in 16 embryos, each of which was observed at least four times over the control period of two hours or more. Since the normal frequencies were found to change by as much as 25% with the age of the organisms, only those data obtained at a given age can be compared strictly. The determinations indicated by crosses are comparable, for a given species, having been obtained over a period not exceeding 10 days. The points shown as circles are also comparable. All remaining observations were made over such a long interval that the change with age was operative and therefore these were not considered in fitting the

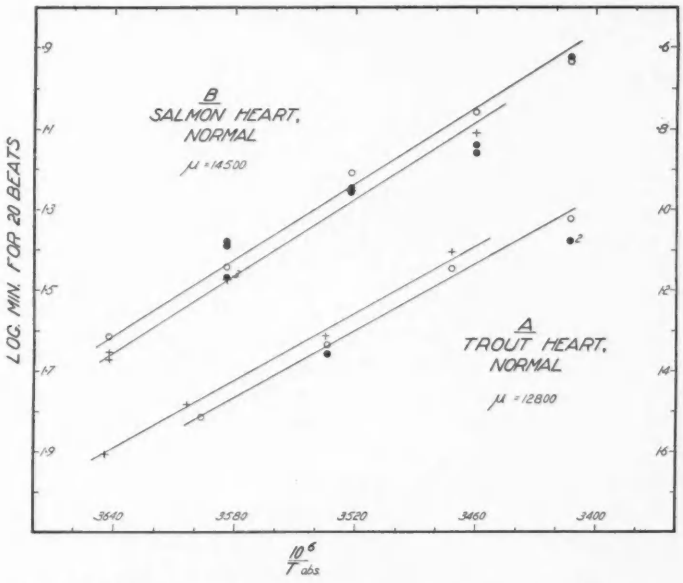


FIG. 6. *The effect of temperature on the normal frequency of the heart-beat in embryos of salmon and trout, respectively. The ordinate at the left of the figure refers to salmon, while that at the right refers to trout.*

straight lines. It is apparent that temperature does not affect the heart-beat frequency in exactly the same way in the two organisms. Moreover, the values of $\mu$ and of $H$ for a given preparation may differ greatly, being, in trout, 12,800 and 23,900, respectively, and in salmon 14,500 and 17,400. Clearly, the circumstances that establish the effect of temperature on the critical partial pressure of oxygen are not identical with those that establish the magnitude of the temperature effect on the normal frequency. Ideally $H$ may be pictured as a measure of the relation between temperature and the concentration of some component in the reaction chain through which oxygen is consumed. On the other hand $\mu$ indicates the effect of temperature on the velocity with which oxygen or its equivalent passes over that chain.

While the rise of the critical oxygen partial pressure with temperature may be a very general phenomenon (5), the critical pressure at a given temperature varies widely from one preparation to another (10). In this connection it is possible to compare the critical pressure for heart-beat frequency in trout and salmon embryos with that in *Fundulus*. For the former at 22° to 23° C. the critical pressure is approximately 60 mm. of mercury while, as judged from the paper by Fisher and Cameron (2), it cannot be greater than about 25 mm. in *Fundulus*.

Though the effect of age was not examined particularly in the present investigation, the data suggest that the critical partial pressure at a given

temperature may rise significantly as the organisms become older. In addition, the whole sequence of events upon exposure to oxygen lack occurs more rapidly as the organisms grow older. These changes with age can undoubtedly be considered as examples of the rather general fact that embryonic organisms are more resistant to asphyxial conditions than are adults.

## Discussion

The precise significance to be attached to the magnitude of the temperature characteristic that is reported here, depends on the mechanism that is responsible for establishing a critical oxygen partial pressure in these preparations. The more obvious of the two conditions that could theoretically lead to the existence of a critical pressure is of course the restriction imposed on oxygen consumption by the physical process of diffusion. In order to be utilized at the centre of a mass of cells oxygen must diffuse through the mass from the source. For a given rate of utilization there may therefore be a limiting partial pressure, the critical pressure, which will just suffice to produce the necessary rate of diffusion. With the rate of utilization determining the critical pressure, the effect of temperature on a critical pressure will be determined to a great degree by the effect of temperature on the rate of utilization.

On the other hand, the existence of a critical oxygen partial pressure as a property of the chemical reactions into which oxygen enters in the cells, is implied (cf. 10) in the quantitative theory of cell respiration developed by Warburg (11). In all respects that have been examined (2) it has been shown that heart-beat frequency can be substituted for oxygen consumption in this theory. It is implied therefore, that the frequency of the heart-beat also should exhibit a critical oxygen pressure, whose significance is identical with that for the critical pressure observed for oxygen consumption. Moreover, the data of Stotz, Altschul, and Hogness (8) show that the isolated cytochrome oxidase system, which is believed to be of very general importance in the respiration of all aerobic cells, exhibits a critical pressure in ranges of oxygen concentration that are physiologically significant.

From the standpoint of cellular respiration the critical pressure is a measure of the affinity of an intracellular oxidative catalyst for oxygen, i.e., a measure of the equilibrium constant ($K$) of the mass law equation applying to the combination of oxygen with the catalyst. The effect of temperature on the critical pressure is thus a measure of the effect of temperature on $K$. This constant, and hence the critical pressure, should be related to temperature by an expression of the van't Hoff isochore (10). That formula is indistinguishable from Equation 1 which, as Figs. 4 and 5 show, can be employed to describe the relation between the critical pressure and the temperature. The data are therefore consistent with the view that the critical pressure is a chemical property of the cellular oxidative systems.

It has not been possible to obtain experimental proof that diffusion either does or does not take part in determining the critical partial pressures that have been observed. However, the organisms used possessed an intact circulatory system so that the actual diffusion distances were probably of the order of the diameter of a single cell. It seems much more likely therefore, that the critical pressures recorded are not due to diffusion but are a property of the chemical mechanism in the pacemaking cells of the heart.

The data described in this paper reveal the precise conditions that must obtain with regard to the partial pressure of oxygen in order that normal functioning of the heart in these embryos may proceed. With the observations in mind, the common practice of lowering the temperature when conditions occur that may be adverse in relation to the oxygen supply, is seen to be advantageous in two respects. It is well known of course that the quantity of oxygen consumed per unit time is smaller, the lower the temperature. It is to be recognized now that lowering the temperature also decreases the critical oxygen partial pressure. As a consequence a larger fraction of the quantity of oxygen present under given conditions can be utilized at low temperatures before effects of oxygen lack occur than can be utilized similarly at higher temperatures. This fact is made particularly striking by a comparison of the actual conditions at two temperatures. From Fig. 5 it can be seen that at 25° C. trout embryos, when placed in water initially saturated with air, could utilize only 30% of the oxygen present before the partial pressure of that gas would be so reduced as to interfere with the heart rate. At 5° C. on the other hand, some 97% of the oxygen could be used before the functioning of the heart would be affected by the low partial pressure.

Though obtained for a particular organ, the heart, these results can probably be taken as indicative of the situation in all organs and, in fact, as characteristic of the entire individual. It should be noted, however, that great distortion of the comparatively simple picture found here is at least theoretically conceivable. If, for example, the passage of oxygen from the external environment involves a respiratory pigment such as haemoglobin (though there was undoubtedly haemoglobin present in the circulatory system of the embryos, it seems unlikely that the pigment played a significant role in the stages used), then the properties of this pigment might result in the appearance of quite a different picture. For such reasons a wide generalization to intact organisms on the basis of the data presented is not possible.

## Acknowledgment

Salmon and trout eggs were obtained from the Fish Culture Branch, Dominion Department of Fisheries, through the courtesy of Mr. J. A. Rodd. The author wishes also to express his indebtedness to Mr. Herbert Pearen for a supply of trout eggs, and his appreciation of the assistance given by Mr. Stephen Clare during the performance of the experiments.

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# THE LIFE CYCLE OF *APANTELES CARPATUS* (SAY) (HYMENOPTERA: BRACONIDAE), A PARASITE OF THE WEBBING CLOTHES MOTH, *TINEOLA BISSELLIELLA* HUM.[1]

By A. Murray Fallis[2]

## Abstract

This parasite has been reared from larvae of the webbing clothes moth obtained from different localities as well as experimentally. All parasites obtained by natural and experimental infections were females. Oviposition occurred and parasites developed in host larvae weighing 1.6 to 6.8 mg. The parasites oviposited more readily in a host enclosed in a case, especially if the case contained fecal pellets of the host. Eggs were deposited in various parts of the host. A single parasite developed to maturity even though several eggs may have been deposited in the host, each by a separate "thrust" of the ovipositor. Morphological features of the larvae are illustrated. The rate of development varied even at constant temperature. The average length of the life cycle at 27° C. was 26 days but at 20° C. it required several months. Experiments were carried out to determine the factors responsible for the variation in the rate of development. The parasite larva, upon emerging from the host, usually spins a white, silken cocoon, although metamorphosis was sometimes completed even though no cocoon was produced.

*Apanteles carpatus* (Say) was reared from the case-making clothes moth, *Tinea pellionella* L. in Connecticut by Mr. W. D. Kearfott in 1905 (Viereck *et al.* (3) ). The present study reports observations on its habits and life history in larvae of the webbing clothes moth, *Tineola bisselliella* Hum. A culture of clothes moths being maintained at the Ontario Research Foundation was almost completely decimated by this parasite. Moth larvae received from three other localities in Toronto were also found to be naturally infected by it.

Mr. Muesebeck of the Bureau of Entomology and Plant Quarantine, Washington, D.C., who kindly identified the parasite, states (personal communication) that it has a world-wide distribution. Viereck *et al.* (3) reported that it also has been bred from the white-marked tussock moth, *Hemerocampa leucostigma* Smith and Abbot, but Muesebeck (personal communication) states, "This is almost certainly incorrect. The species is not infrequently reared from clusters of tussock moth cocoons, not as a parasite of that lepidopteran but of one of the several tineid scavengers that occur in such situations."

## Habits of Adults

Adult parasites were kept at approximately 20° C. and fed on diluted honey. Each was placed in a separate vial in the bottom of which was absorbent cotton, partly moistened with honey. A large number were kept alive in vials for two to three weeks, although some died much sooner. Many of the parasites drank thirstily from drops of water when they were made available to them.

[1] *Manuscript received July 18, 1941.*

*Contribution from the Department of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Ont.*

[2] *Research Fellow.*

Adults (Fig. 1) are attracted to light of certain intensities, especially just after emergence from the cocoon, for they invariably flew to the window when they escaped. The antennae are held straight in front when the insect is resting but they are curved ventrally when it moves about. The ovipositor can be seen protruding from the posterior end of the abdomen. The characteristic fore wing is illustrated in Fig. 2.

These insects are excitable and easily disturbed. At times they showed no inclination to oviposit, although a short time later they might attempt to do so. The ovipositor is illustrated in Fig. 3. This photograph shows a lateral view of the abdomen of an insect which was cleared and mounted in balsam. The stylets of the ovipositor as well as the sheaths are visible. The stylets are held within the sheaths when the insect is at rest but are sharply bent ventrally during oviposition so that the extremities of the sheaths move toward the base of the stylets and appear to act as a guide for them.

## Oviposition

Eggs are produced parthenogenetically. The sense of odour or touch, or both, appeared more effective in directing the parasite to a host than the sense of sight, for the host frequents dark places yet, as previously mentioned, the parasite is attracted to light. Moreover, moth larvae were attacked more readily if encased, especially if fecal pellets had been used in the construction of the cases. "Thrusts" were made even into empty cases and frequently at a single fecal pellet. Only a few seconds were required for oviposition, which took place in any part of the host. A parasite would deposit several eggs in the same host, a separate "thrust" of the ovipositor being required to deposit each egg, although sometimes no egg was left. The "thrusts" sometimes caused the larva within a case to emerge. All the eggs usually hatched but of the resulting larvae only one grew to maturity.

The maximum lengths of living larvae found in the same host were 0.5 mm. and 0.3 mm., respectively, but in most cases by the time one of the larvae had

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### Explanation of Figures

#### Plate I

*All figures represent stages in the life cycle of Apanteles carpatus (Say).*

Fig. 1. *Adult. ca. 5×.*
Fig. 2. *Fore wing of adult. ca. 16×.*
Fig. 3. *Lateral view of abdomen to show ovipositor. ca. 16×.*
Fig. 4. *Appearance of egg when deposited in host. ca. 190×.*
Figs. 5, 6, 7. *Successive stages in development of eggs. ca. 190×.*
Fig. 8. *Ventral view of larva shortly after emerging from egg. ca. 190×.*
Fig. 9. *Lateroventral view of larva 0.5 mm. long. ca. 110×.*
Fig. 10. *Lateral view of larva 0.5 mm. long. ca. 110×.*
Fig. 11. *Laterodorsal view of larva 0.6 mm. long. ca. 110×.*
Fig. 12. *Lateral view of larva 1.5 mm. long. ca. 60×.*
Fig. 13. *Silken cocoon from which parasite has emerged. Note cap removed from one end. ca. 4×.*

PLATE I


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2


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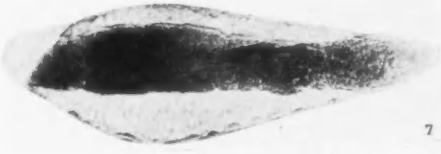
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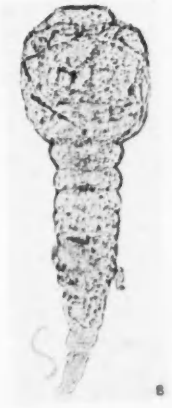
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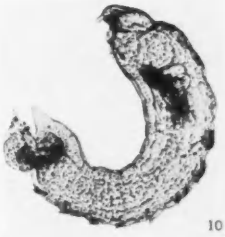
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reached a length of 0.5 mm., the others were all dead. Eggs may be laid by a parasite the same day that it has emerged from a cocoon.

## Development and Morphology of Larvae

The size of the eggs (Fig. 4) immediately following oviposition, based on a measurement of 10 eggs, is: average length—0.28 mm., maximum—0.30 mm., minimum—0.24 mm.; average width of expanded portion—0.035 mm., maximum—0.044 mm., minimum—0.024 mm. Development of the egg is rapid (Figs. 5, 6, 7) and a larva (Fig. 8) hatches in a few days, the actual rate depending on the temperature.

Internal structure in the first stage larva is not very apparent. The broad cephalothoracic region compared to the narrower abdominal region is noticeable. A strong pair of mandibles is attached to the ventral surface just ventral to the oral aperture. The so-called antennae (Fig. 14), which are anterior and dorsal to the mandibles, are small, movable, teat-like structures, with a small spine near the base of the dorsal surface of each. An irregular number of spines are arranged in rows on the dorsal surfaces of the eight (occasionally, nine) posterior abdominal segments (Fig. 14). A long caudal appendage is also apparent.

Larvae, 0.5 to 0.6 mm. in length, are shown in Figs. 9, 10, 11. The mandibles and antennae are both distinguishable in Fig. 9, but the shape and relative position of the antennae are shown to better advantage in Fig. 10. A prominent caudal vesicle is noticeable. The caudal appendage appears relatively smaller than in the earlier stage. The position of the brain and gonads may also be seen in Fig. 10, although they are better illustrated in Fig. 11. These various structures will be located more readily by reference to Figs. 14 and 15.

The brain, nerve cord, intestine, heart, and ovaries are well developed in larvae 1.5 mm. long or even smaller (Figs. 12 and 15). The heart and nerve cord were observed in some specimens 0.7 to 0.8 mm. long. The silk glands and ducts (Fig. 15) are also formed, although the former become more extensive later. Larvae measured 3 to 4 mm. in length upon emergence from the hosts (Fig. 17) and in those 3 mm. long (Fig. 16) the main branches of the tracheal system are visible. The mouth parts are more difficult to distinguish than in smaller forms. The mandibles are relatively smaller and, on the posteroventral border of each, there is a row of small teeth. The silk glands are extensive. The anlages of some of the appendages are visible in stained specimens. Minute spines occur over the entire body.

## Rate of Development

The rate of development of the parasite varies even at a constant temperature. The minimum, maximum, and average time, at 27° C., between the deposition of eggs in 81 moth larvae and the emergence of mature parasite larvae from these hosts was 13, 45, and 20 days, respectively. The minimum

time at 24° C. was 20 days, the maximum, 154. The average for this temperature is not given, as only six parasites were reared and, although five of them emerged from their hosts in approximately 30 days, the sixth took much longer. Development at room temperature requires several months.

The growth of some larvae at 27° C. was as rapid as that shown in Table I. This is probably an extreme rate, the average being somewhat slower.

TABLE I

GROWTH OF LARVAE

| Time, days | Size of larvae, mm. | Time, days | Size of larvae, mm. |
|---|---|---|---|
| 1 | Egg developing | 5 | 0.48 |
| 2 | Egg developing | 6 | 0.72 |
| 3 | 0.36 | 7 | 1.52 |
| 4 | 0.40 | 10 | 4.0 |

The average time spent in the pupa stage (including the prepupa of some authors) at 27° C. was eight days for 40 individuals, at 24° C., 11 days for five individuals, and at 20° C. (approximately), 17 days for three. Therefore, at 27° C. the life history should be completed in an average of 28 days. However, only 44 of the parasites completed their metamorphosis successfully following pupation, and the average length of their complete cycle at 27° C. was 26 days. This means that more of those parasites completed their cycle when the larval period was short than when it was more than the average length.

The parasite, after emerging from its host, spins a white, silken cocoon (Fig. 13) within the case in which the host is lying. Any attempt to spin a cocoon when the host is not within a case is unsuccessful, apparently because of the absence of a framework about it to assist in the process. It is suggested by these studies that a larger percentage of the parasites that pupate within host cocoons succeed in metamorphosing to adults than do those that are not in cocoons. Over 50% of the former changed to adults whereas only one out of six of the latter did so. The imago escapes from the cocoon by cutting a symmetrical opening at one end and then pushing the cap thus formed (Fig. 13) out of its way.

EXPLANATION OF FIGURES

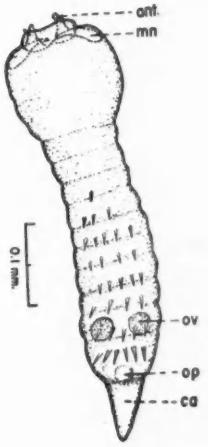
Fig. 14. *Outline drawing of first larval stage, ventral view.*

Fig. 15. *Drawing, somewhat diagrammatic, of 1.5 mm. larva, lateral view.*

Fig. 16. *Main branches of tracheal system of 3 mm. larva as seen in lateral view.*

Fig. 17. *Drawing, somewhat diagrammatic, of 3 mm. larva, ventral view.*

*Abbreviations: anl., anlage of appendages; ant., antenna; br., brain; ca., caudal appendage; cv., caudal vesicle; com., circumoesophageal commissure; int., intestine; md., mn., mandible; nc., nerve cord; op., opening for caudal vesicle; or., oral aperture; ov., gonad; sd., silk duct; sg., silk gland.*


ant.
mn
0.1 mm.
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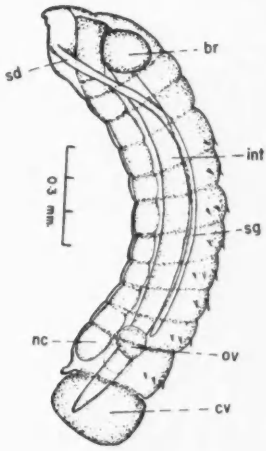
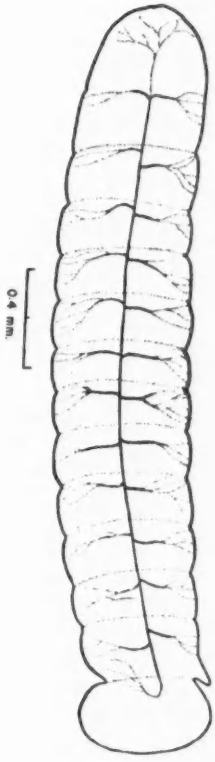
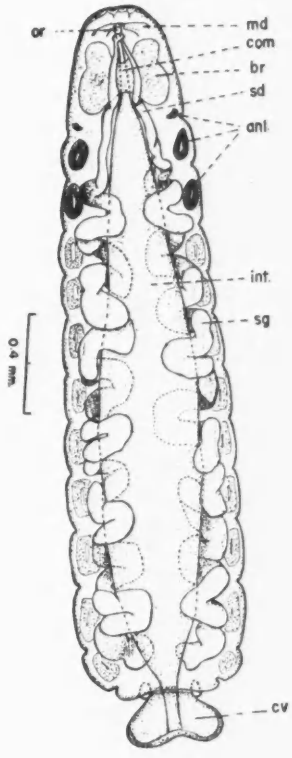
14


br
sd
int
0.3 mm.
sg
nc
ov
cv

15


0.4 mm.

16


or
md
com
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sd
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sg
0.4 mm.
cv

17

Parasites were reared from hosts weighing 1.6 to 6.8 mg. There was no indication from experimental infections that development was faster in the larger hosts. There was no appreciable difference in the rate of development of parasites in hosts that had received several "thrusts" of the ovipositor as compared to those that received a single "thrust". Parasitic larvae emerged in an average of 19 days from 22 hosts in the former group and in an average of 18 days from 34 hosts in the latter group. It is worth noting that Moss (2) found that *Apanteles glomeratus* L. developed as rapidly in a host containing 100 parasites as in one that contained only 20.

The average time from deposition of the eggs until the emergence of parasites from 15 hosts receiving one "thrust" of the ovipositor in the anterior region of the body was 21 days, whereas it was only 17 days in the case of nine hosts that received one "thrust" in the posterior part of the body. This difference may not be significant as the eggs were not all deposited by the same parasite and it will be seen from Table II that the average rate of development of the progeny from one parent may differ from that of another.

TABLE II

AVERAGE RATE OF DEVELOPMENT OF PROGENY OF FIVE PARASITES

| — | Parasite No. | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Number of hosts from which parasites emerged | 8 | 9 | 7 | 8 | 12 |
| Time (days) until emergence of parasite larvae | | | | | |
| Minimum | 13 | 14 | 14 | 13 | 14 |
| Maximum | 53 | 31 | 19 | 30 | 22 |
| Average | 24 | 18 | 16 | 24 | 17 |

The table includes only those hosts from which larvae emerged. In many cases the host died before the parasites had completed their development. The hosts used in the experiments received "thrusts" by the parasites in different parts of their bodies. The results of this small experiment suggest that the average rate of development of a parasite may be partially dependent on its parentage.

Environmental humidity as well as temperature may affect development of the parasites (Table III).

TABLE III

DEVELOPMENT AT 27° C. AND THREE DIFFERENT RELATIVE HUMIDITIES

| — | Relative humidity, % | | |
|---|---|---|---|
| | 30 | 45 | 75 |
| Number of hosts infected | 22 | 28 | 15 |
| Percentage of hosts from which larvae emerged | 77 | 57 | 60 |
| Percentage of hosts from which adult parasites were obtained | 59 | 28 | 26 |
| Percentage of emerging larvae metamorphosing to adults | 76 | 50 | 44 |

A relative humidity of 30% would appear, therefore, to be the more suitable for the development of the larvae as well as for metamorphosis into the adult parasite. The humidity might be expected to have the most influence on the pupal stage but the effect on larval development appears from the above to be equally marked. The effect of humidity on the parasite thus seemed to differ from that on the host itself, for Griswold and Crowell (1) found a relative humidity of 75% most favourable for the development of the webbing clothes moth larvae.

## Acknowledgments

The author would like to acknowledge gratefully the kind assistance and co-operation of those who have made this work possible—Dr. H. B. Speakman, Director of the Foundation, for his advice and encouragement; Dr. S. Hadwen under whom the work was carried out; Dr. E. M. Walker for assistance in interpreting the morphology; Mr. C. F. W. Muesebeck for identifying the parasite, making helpful suggestions and reading the manuscript; Mr. Norman Law for assisting with many of the experiments and photographs.

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# ON THE USE OF THE pH VALUE AS A MEASURE OF THE FRESHNESS OF FISH MUSCLE TISSUE[1]

By F. Charnley[2] and D. H. Goard[3]

## Abstract

The pH value of the aqueous liquors derived from fish muscle tissue is connected through statistical relations with the buffer action of the liquor, the log bacterial count of the sample, and with a subjective estimate of freshness determined on the basis of odour. In the case of pH and odour rating the relation is not a correlation but, instead, a linear relation between the means of a series of populations. By means of the latter it is possible from observations of the pH of the aqueous liquor in the sample to determine objectively the freshness of a parcel of canned chum salmon to any desired degree of accuracy by increasing the size, *n*, of the sample taken for examination.

The pH value possesses a number of advantages over other tests that have been proposed from time to time for measuring spoilage in fish or animal tissue. One of these is that the pH can be measured accurately; secondly, the test can be carried out rapidly; and thirdly, the pH, owing to the fact that it is apparently a function of the degradation or fission of all protein constituents in the tissue, is the most generally applicable of any of the chemical tests so far suggested for this purpose. As in other tests for spoilage, however, the pH of fish muscle tissue, or aqueous liquors derived therefrom, shows considerable variation from sample to sample, so that before the test can be utilized for the accurate measurement of spoilage, it is necessary to know not only the relation between the pH value and a criterion of freshness, but also the standard deviation of the pH in samples drawn from the same quality as regards freshness.

The two fundamental criteria available for testing the validity of the pH as a measure of freshness consist of (1) a chemical test based on the observation of Van Slyke, that, as primary changes proceed, the buffer capacity of the tissue decreases, and (2) bacteriological tests, in which the pH value is compared with bacterial counts as deterioration proceeds. In addition to the two fundamental criteria, however, there is a third and, practically, more important criterion than either of these for testing the validity of the pH as a measure of freshness, namely, the ratings of an experienced examiner based on the results of organoleptic tests, that is, on odour and other evidences of incipient decomposition. The investigation reported in this paper was concerned primarily with the relation between pH and this third criterion.

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## Relations between pH, Buffer Action, and Log Bacterial Count

The nature of the relations between the pH value of an aqueous suspension of the sample and the first two criteria of freshness can be readily illustrated by the results of Stansby and Griffiths (6). In their study of the deterioration in quality of freshly caught haddock these authors made bacterial counts on the samples and measured the buffer capacity of a suspension of 5 gm. of the muscle tissue in 100 cc. of water. The number of cubic centimetres of 0.0165 *N* hydrochloric acid required to bring the pH of the suspension to 6 was taken as a measure of the secondary changes in the fish tissue and was termed the *B* value, while the additional number of cubic centimetres of the acid required to bring the pH to about 4 was taken as a measure of the primary

TABLE I

RELATION BETWEEN pH AND $100/A$

| pH | $100/A$ | | | | | | | | | | | | | | *f* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3.06 to 3.55 | 3.56 to 4.05 | 4.06 to 4.55 | 4.56 to 5.05 | 5.06 to 5.55 | 5.56 to 6.05 | 6.06 to 6.55 | 6.56 to 7.05 | 7.06 to 7.55 | 7.56 to 8.05 | 8.06 to 8.55 | 8.56 to 9.05 | 9.06 to 9.55 | 9.56 to 10.05 | |
| 7.41 to 7.47 | | | | | | | | | | | | | | 1 | 1 |
| 7.34 to 7.40 | | | | | | 2 | | 1 | | | | | | | 3 |
| 7.27 to 7.33 | | | | | | | | | | | | | | | – |
| 7.20 to 7.26 | | | | | 1 | 1 | | | | | | | | | 2 |
| 7.13 to 7.19 | | | | | | 1 | 1 | | 1 | | | | | | 3 |
| 7.06 to 7.12 | | | | 1 | 4 | | | 1 | 1 | | | | | | 7 |
| 6.99 to 7.05 | | | 2 | 1 | 1 | 1 | 1 | 1 | | | | | | | 7 |
| 6.92 to 6.98 | | | 1 | 2 | 1 | 1 | 2 | | | | | | | | 7 |
| 6.85 to 6.91 | 1 | | 2 | 2 | | 2 | 1 | | | | 1 | | | | 9 |
| 6.78 to 6.84 | | | 4 | 3 | | | | | | | | | | | 7 |
| 6.71 to 6.77 | | 3 | 3 | 1 | | | | | | | | | | | 7 |
| 6.64 to 6.70 | | 1 | 2 | 2 | 1 | | | | | | | | | | 6 |
| 6.57 to 6.63 | | | 3 | | | | | | | | | | | | 3 |
| 6.50 to 6.56 | | | 1 | | | | | | | | | | | | 1 |
| *f* | 1 | 4 | 18 | 12 | 8 | 8 | 5 | 3 | 2 | – | 1 | – | – | 1 | 63 |

NOTE: $M_1 = 5.389$; $M_2 = 6.921$; $S_X = 1.156$; $S_Y = 0.2063$; $R = 0.6363$.

changes that had taken place in the sample. This was called the $A$ value and was based on the results of Van Slyke (7).

Evidently, only the $A$ value can properly be regarded as a measure of changes in the constituents of the muscle tissue, since the $B$ value refers to different pH ranges and will tend merely to follow the titration curve. The two quantities most suitable for revealing the character of the relation between the pH value and freshness are therefore the $A$ value and the log bacterial count. Since, however, the $A$ value decreases with increasing pH value, it was found more convenient to employ the quantity $100/A$, that is, a measure of the buffer action (1) instead of the buffer capacity of the suspension.

Tables I, II, and III show the relations between the three quantities,

TABLE II

RELATION BETWEEN pH AND LOG BACTERIAL COUNT

| pH | Log bacterial count | | | | | | | | | | | $f$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2.8 to 3.2 | 3.3 to 3.7 | 3.8 to 4.2 | 4.3 to 4.7 | 4.8 to 5.2 | 5.3 to 5.7 | 5.8 to 6.2 | 6.3 to 6.7 | 6.8 to 7.2 | 7.3 to 7.7 | 7.8 to 8.2 | |
| 7.41 to 7.47 | | | | | | | | 1 | | | | 1 |
| 7.34 to 7.40 | | | | | | | | | 2 | | | 2 |
| 7.27 to 7.33 | | | | | | | | | | | | – |
| 7.20 to 7.26 | | | | | | | | 1 | | | 1 | 2 |
| 7.13 to 7.19 | | | | | 1 | | | | 1 | | | 2 |
| 7.06 to 7.12 | | | | | | 2 | 1 | 2 | | | | 5 |
| 6.99 to 7.05 | | | | | | 1 | | 2 | 1 | | | 4 |
| 6.92 to 6.98 | | | | 1 | 1 | 2 | 1 | 1 | 1 | | | 7 |
| 6.85 to 6.91 | 1 | | 1 | 1 | | 4 | 1 | | | 1 | | 9 |
| 6.78 to 6.84 | 1 | | 3 | 2 | | | 1 | | | | | 7 |
| 6.71 to 6.77 | 1 | 1 | 1 | | 2 | 1 | | | 1 | | | 7 |
| 6.64 to 6.70 | | | | | 1 | 5 | 1 | | | | | 7 |
| 6.57 to 6.63 | | | 1 | | | 1 | 1 | | | | | 3 |
| 6.50 to 6.56 | | | | | | 1 | | | | | | 1 |
| $f$ | 3 | 1 | 6 | 4 | 5 | 17 | 6 | 7 | 6 | 1 | 1 | 57 |

NOTE: $M_1 = 5.474$; $M_2 = 6.894$; $S_X = 1.137$; $S_Y = 0.2033$; $R = 0.4796$.

pH, log bacterial count, and $100/A$ derived from the data of Stansby and Griffiths. From an inspection of these data, it is apparent that there are definite relations between these three variates. As measured by the values of the correlation coefficient, the relations involving bacterial count are not as close as might have been expected, but that connecting pH and $100/A$ shows a relatively close correspondence between the two variates.

In the data of Tables I, II, and III the usual significance cannot be attached to the values of $R$, since there is no evidence that the samples have been drawn from the same quality as regards freshness. In fact, by virtue of the differences in treatment of the various sets of samples, it may be safely inferred that the samples represent varying degrees of quality in this respect. Hence, the data are very probably composite distributions and the values of the correlation coefficient only apparent correlations. The data, unfortunately, are not sufficiently numerous to allow a satisfactory investigation of the true character of the relations between the three variates, but in the

TABLE III

RELATION BETWEEN $100/A$ AND LOG BACTERIAL COUNT

| $100/A$ | Log bacterial count | | | | | | | | | | | $f$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2.8 to 3.2 | 3.3 to 3.7 | 3.8 to 4.2 | 4.3 to 4.7 | 4.8 to 5.2 | 5.3 to 5.7 | 5.8 to 6.2 | 6.3 to 6.7 | 6.8 to 7.2 | 7.3 to 7.7 | 7.8 to 8.2 | |
| 8.06 to 8.55 | | | | | | 1 | | | | | | 1 |
| 7.56 to 8.05 | | | | | | | | | | | | - |
| 7.06 to 7.55 | | | | | | | | 1 | 1 | | | 2 |
| 6.56 to 7.05 | | | | | | | | 2 | 1 | | | 3 |
| 6.06 to 6.55 | | | | | 1 | 1 | 1 | 1 | 1 | | | 5 |
| 5.56 to 6.05 | | | | 1 | | 2 | | 2 | 2 | 1 | | 8 |
| 5.06 to 5.55 | | | | | 1 | 4 | 1 | | 1 | 1 | 1 | 9 |
| 4.56 to 5.05 | 1 | | 1 | 1 | | 4 | 3 | 1 | 2 | | | 13 |
| 4.06 to 4.55 | 1 | | 5 | 2 | 1 | 7 | 1 | | | | | 17 |
| 3.56 to 4.05 | | 1 | | | 2 | 1 | | | | | | 4 |
| 3.06 to 3.55 | 1 | | | | | | | | | | | 1 |
| $f$ | 3 | 1 | 6 | 4 | 5 | 20 | 6 | 7 | 8 | 2 | 1 | 63 |

NOTE: $M_1 = 5.556$; $M_2 = 5.125$; $S_X = 1.141$; $S_Y = 0.9926$; $R = 0.5270$.

case of the variates pH and $100/A$, the relation appears to be very similar to that connecting the pH value and examiner's rating discussed below.

## Relation between pH and Examiner's Rating

The results employed in determining the relation between the pH value and examiner's rating for freshness were derived from samples of canned chum salmon (*Oncorhynchus Keta* Walbaum, 1792) packed late in the season between September 15 and October 31, 1936. The pH values are the readings given by the aqueous liquid in the samples and were determined by means of a Beckman glass electrode apparatus. The examiner's ratings were, as far as possible, based on the odour of the interior of the sample. These were determined immediately before the pH value of the aqueous liquid in the sample was taken and were recorded as follows: $A$ = good, $B$ = better than average, $C$ = average, $D$ = stale, and $E$ = tainted.

Table IV shows the distributions of the pH of the free aqueous liquid in the various samples tabulated according to packing date. On assigning the

TABLE IV

DISTRIBUTION OF pH OF FREE AQUEOUS LIQUID IN SAMPLES OF CANNED CHUM SALMON PACKED BETWEEN SEPT. 15 AND OCT. 31, 1936, IN THE FRASER RIVER AND VANCOUVER ISLAND AREAS

| pH | Packing date | | | | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | September | | | | | October | | | | | | | | | | | | |
| | 15 | 18 | 19 | 24 | 25 | 11 | 14 | 15 | 16 | 17 | 22 | 23 | 24 | 27 | 28 | 30 | 31 | |
| 6.61 to 6.63 | | | | | | | | | | | | 1 | | | | | | 1 |
| 6.58 to 6.60 | | | | | | | | | | | | | | | | | | |
| 6.55 to 6.57 | | | | | | | | | | | | 1 | | | | | | 1 |
| 6.52 to 6.54 | | | | | | | | | | | | 1 | 1 | | | | | 2 |
| 6.49 to 6.51 | | | | | | | | | | | | | | | | | | |
| 6.46 to 6.48 | | | | | | | | | | | 1 | 1 | | 2 | 1 | | | 5 |
| 6.43 to 6.45 | 2 | | | | | | | | 1 | 1 | 2 | 3 | | | 2 | | | 11 |
| 6.40 to 6.42 | | 1 | | | | | 1 | | | | 2 | 4 | 3 | 1 | | | | 12 |
| 6.37 to 6.39 | | | | | 3 | | 2 | 2 | 1 | 1 | 3 | 1 | 2 | | | | 1 | 16 |
| 6.34 to 6.36 | | | | | 1 | | 2 | 1 | 4 | 4 | 4 | | 1 | | | | | 17 |
| 6.31 to 6.33 | | | 1 | | 3 | | 1 | 2 | 4 | 6 | 2 | | 1 | | | | | 20 |
| 6.28 to 6.30 | | | | | 2 | 2 | 2 | 2 | 7 | 5 | 1 | 1 | 1 | 1 | | | | 24 |
| 6.25 to 6.27 | | 1 | | | 5 | 1 | | | 4 | 3 | 1 | | 2 | | | 1 | | 18 |
| 6.22 to 6.24 | | | | 2 | 3 | | 1 | 2 | | 2 | | | | | | | | 10 |
| 6.19 to 6.21 | | | | 1 | 1 | | | | | 2 | | | | | | | | 4 |
| Total | 2 | 2 | 1 | 3 | 18 | 3 | 9 | 9 | 21 | 24 | 16 | 13 | 11 | 4 | 3 | 1 | 1 | 141 |

numbers $A = 1$, $B = 2$, etc., to the examiner's ratings, the corresponding distributions of examiner's ratings shown in Table V are obtained. From an inspection of the data in these two tables, it will be observed that in both instances there is a suggestion of a seasonal trend. As they stand, however, the data are almost useless for practical grading purposes since, in the first place, the trends are incomplete, secondly, the trends, if any, cannot be

TABLE V

DISTRIBUTION OF ODOUR RATING IN SAMPLES OF CANNED CHUM SALMON PACKED BETWEEN SEPT. 15 and OCT. 31, 1936, IN THE FRASER RIVER AND VANCOUVER ISLAND AREAS

| Odour rating | Packing date | | | | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | September | | | | | October | | | | | | | | | | | | |
| | 15 | 18 | 19 | 24 | 25 | 11 | 14 | 15 | 16 | 17 | 22 | 23 | 24 | 27 | 28 | 30 | 31 | |
| 5 | | | | | | | | | | | | 8 | | 2 | | | | 10 |
| 4 | 2 | 2 | 1 | | | | 1 | | 6 | 7 | | 2 | 4 | 2 | 3 | 1 | 1 | 32 |
| 3 | | | | | 2 | 3 | 7 | 7 | 11 | 9 | 13 | 3 | 7 | | | | | 62 |
| 2 | | | | 3 | 16 | | 1 | 2 | 4 | 8 | 3 | | | | | | | 37 |
| Total | 2 | 2 | 1 | 3 | 18 | 3 | 9 | 9 | 21 | 24 | 16 | 13 | 11 | 4 | 3 | 1 | 1 | 141 |

adequately described by means of simple regression functions, and, thirdly, even if the regression functions were known, there is no evidence to show that such trends will be exactly repeated in future, or that samples packed in other districts by other canneries will be exactly described by these trends. To obtain data that will be suitable for practical grading purposes, therefore, the relation connecting the variates pH and odour rating will be required.

TABLE VI

RELATION BETWEEN EXAMINER'S RATING (ODOUR) AND pH OF AQUEOUS LIQUID IN SAMPLES OF CANNED CHUM SALMON

| pH ($Y$) | Odour ($X$) | | | | $f$ |
|---|---|---|---|---|---|
| | $B$ 2 | $C$ 3 | $D$ 4 | $E$ 5 | |
| 6.60 to 6.649 | | | | 1 | 1 |
| 6.55 to 6.599 | | | | 1 | 1 |
| 6.50 to 6.549 | | | 1 | 1 | 2 |
| 6.45 to 6.499 | | 2 | 7 | 2 | 11 |
| 6.40 to 6.449 | | 8 | 6 | 3 | 17 |
| 6.35 to 6.399 | 8 | 15 | 3 | 1 | 27 |
| 6.30 to 6.349 | 14 | 17 | 7 | 1 | 39 |
| 6.25 to 6.299 | 7 | 15 | 7 | | 29 |
| 6.20 to 6.249 | 7 | 5 | 1 | | 13 |
| 6.15 to 6.199 | 1 | | | | 1 |
| $f$ | 37 | 62 | 32 | 10 | 141 |

NOTE: $M_1 = 3.1064$; $M_2 = 6.3445$; $S_X = 0.8728$; $S_Y = 0.07940$; $R = 0.4971$.

Table VI shows values of pH tabulated according to odour rating. Comparison of the data listed in this table with the example of a linear composite bivariate normal distribution given by Charnley (2) suggests that the distribution relating pH and odour rating is also of the latter type.

If this hypothesis is correct, then from the data given in Table VI it is immediately evident that the slope $m$ of the line relating the means of the component distributions is greater than zero. Consequently, if the variance in pH estimated from the individual codes, that is, from samples packed by the individual canneries on different days, is compared with the variance in pH given by the composite distribution (Table VI), there should be a significant difference in the two values.

The details of the subgroups from which the variance in pH in the individual populations was estimated for the purpose of this comparison are shown in Table VII. As will be seen from the table, the samples were packed at

TABLE VII

SUMS OF SQUARES OF pH AND ODOUR RATING IN INDIVIDUAL SUBGROUPS

| Company | Cannery | Packing date | Sample size | $nS_Y^2$ | $\Sigma XY$ | $nS_X^2$ |
|---|---|---|---|---|---|---|
| *A* | I | Sept. 15 | 2 | 0.00005 | 0..... | 0..... |
| | I | Sept. 18, 19 | 3 | 0.01447 | 0..... | 0..... |
| | I | Sept. 24 | 3 | 0.00127 | 0..... | 0..... |
| | I | Sept. 25 | 3 | 0.00027 | 0..... | 0..... |
| | I | Oct. 11 | 3 | 0.00127 | 0..... | 0..... |
| | I | Oct. 14 | 9 | 0.02996 | −0.02000 | 2.0000 |
| | I | Oct. 15 | 9 | 0.02762 | −0.00672 | 1.5556 |
| | I | Oct. 16 | 9 | 0.02960 | 0.06452 | 2.2224 |
| | I | Oct. 17 | 12 | 0.01609 | 0.06322 | 2.6668 |
| | I | Oct. 22 | 6 | 0.01393 | 0.02180 | 1.3334 |
| | I | Oct. 23 | 3 | 0.00127 | 0..... | 0..... |
| | I | Oct. 24 | 3 | 0.00006 | 0..... | 0..... |
| | I | Oct. 27 | 2 | 0.02000 | 0..... | 0..... |
| | I | Oct. 28 | 3 | 0.00080 | 0..... | 0..... |
| | I | Oct. 30, 31 | 2 | 0.00500 | 0..... | 0..... |
| Total | | | 72 | 0.16166 | 0.12282 | 9.7782 |
| *B* | II | Sept. 25 | 15 | 0.03198 | −0.03813 | 1.7335 |
| | II | Oct. 16 | 12 | 0.00769 | 0.01100 | 3.0000 |
| | II | Oct. 17 | 12 | 0.05520 | −0.01960 | 2.9168 |
| | III | Oct. 22 | 10 | 0.02285 | −0.00300 | 0.9000 |
| | III | Oct. 23 | 10 | 0.08521 | 0.13480 | 1.6000 |
| | III | Oct. 24 | 8 | 0.02640 | 0..... | 2.0000 |
| | III | Oct. 27 | 2 | 0.00245 | 0..... | 0..... |
| Total | | | 69 | 0.23178 | 0.08507 | 12.1503 |
| *A* + *B* | | | 141 | 0.39344 | 0.20789 | 21.9285 |

three different canneries, two of the latter being operated by the same company. On applying Pearson's formula (4), namely,

$$\sigma_i^2 = \frac{1}{N-k} \sum_{t=1}^{k} \sum_{i=1}^{n_t} (x_{it} - \bar{x}_t)^2 ,$$

to the sum of the squares of the second variate (pH) given in Table VII it is found that $\sigma_y^2 = 0.003306$ pH units$^2$. Hence, if for convenience the scale of the second variate is so changed that 0.05 pH unit = 1 unit in the new scale, $\sigma_y^2 = 1.3225$.

The composite character of the distribution given in Table VI is now easily demonstrated by applying Sheppard's correction to the value of $S_Y^2$ calculated from the combined data and comparing (3) the two variances $S_Y^2$, $\sigma_y^2$, as shown in Table VIII. The result $P = 0.0005$ shows very definitely that the distribution of pH and odour rating given in Table VI is a composite distribution.

TABLE VIII

COMPARISON OF STANDARD DEVIATIONS IN pH ESTIMATED FROM INDIVIDUAL SUBGROUPS AND FROM COMBINED DATA

| Distribution | D.f. ($n$) | Mean square | Log$_e$ S.D. | $1/n$ |
|---|---|---|---|---|
| Single | 119 | 1.3225 | 0.1398 | 0.008403 |
| Composite | 140 | 2.4383 | 0.4457 | 0.007143 |
| | | | −0.3059 | 0.015546 |

NOTE: $z = -0.3059$; $\sigma_z = 0.08816$; *normal var.* = 3.4698; $P = 0.00052$.

Owing to the very broad categories in which the examiner's ratings occur, the component populations in the composite distribution given in Table VI differ considerably from normal with respect to the independent variate (examiner's ratings). Hence, we cannot legitimately estimate $r$ by Fisher's (3) method, that is, by weighting corresponding values of $z$ in the individual subgroups inversely as their variances. Also, for the same reason, the equation (2),

$$\mu_4' - m^4\mu_4 = 3(\mu_2')^2 - 3m^4(\mu_2)^2$$

cannot be safely applied to these data for calculating the value of $m$, the slope of the line of relation. The formulae given by Charnley (2), however, for calculating $m$ and $S_{YL}^2$, the vertical variance around the line of relation, are rigidly applicable to all types of linear, composite, bivariate distributions, so that we may estimate the value of $m$ and test the hypothesis regarding the linearity of the data of Table VI independently of any assumptions concerning the functional form of the data.

The equation giving the value of $m$ is

$$\frac{S_Y^2 - \sigma_y^2}{S_X^2 - \sigma_x^2} = m^2 ,$$

in which $S_X^2$, $S_Y^2$, $\sigma_x^2$, and $\sigma_y^2$ are, respectively, the variances of the composite and component distributions. Applying Pearson's formula to the sum of the squares for odour rating given in Table VII we obtain as an estimate of the variance of the odour rating in the individual subgroups, $\sigma_x^2 = 0.1843$. The numerical values required in calculating $m$ from the above equation are

accordingly, $S_X^2 = 0.7617$, $S_Y^2 = 2.4383$, $\sigma_x^2 = 0.1843$, and $\sigma_y^2 = 1.3225$. The substitution of these values in the equation gives $m^2 = 1.9325$ so that $m = 1.3901$. Expressed in the new units the line of relation is therefore a line passing through the point (0.1064, 0.3901) and having the slope $m = 1.3901$. The position of this line relative to the distribution given in Table VI is shown graphically in Fig. 1.

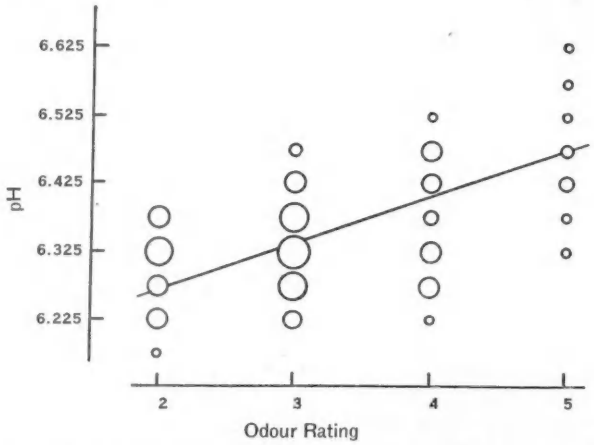


FIG. 1. *Diagram showing line of relation calculated from the data of Table VI. Areas of circles are approximately proportional to corresponding frequencies of single measures.*

## Test of Linearity of Relation Between pH and Odour Rating

In view of the broad categories in odour rating and the consequent lack of normality of this variate in the component populations, the safest procedure in testing the hypothesis as regards linearity is to test averages of samples drawn from the individual subgroups. Averages, even of low sample sizes, such as two or three, will be more nearly normal than the single measures and in this case will probably be sufficiently close to normal to give a reasonably reliable test of the hypothesis, whether we apply Fisher's method of analysis of variance to the variance around the line of relation or set sampling limits on the averages.

The latter method was chosen for testing the averages of samples drawn from the individual subgroups listed in Table VII, since by applying Tchebycheff's inequality (5) this method can be made independent of the nature of the functional forms of the distributions of single measures or averages around the line of relation. To obtain reliable results by this method, however, it is necessary to know that the individual subgroups are reasonably homogeneous as regards the variances of the two variates and the correlation between the variates.

To ensure that this last condition was approximately met, the data of Table VI were therefore segregated according to companies. From Table IX it will be seen that the two groups of data are definitely non-homogeneous as regards variance in pH among single samples. Accordingly, to test the linearity of the relation between pH and odour rating, sampling limits were applied separately to the two sets of averages derived from the individual companies.

TABLE IX

TEST OF HOMOGENEITY OF VARIANCE IN pH IN SAMPLES PACKED BY COMPANIES *A* AND *B*

| Company | D.f. | Sum of squares | Mean square | $\text{Log}_e$ S.D. | $\frac{1}{n}$ |
|---|---|---|---|---|---|
| *A* | 57 | 0.1617 | 0.002837 | −0.6299 | 0.01754 |
| *B* | 62 | 0.3934 | 0.006345 | −0.2274 | 0.01613 |
| | | | | −0.4025 | 0.03367 |

NOTE: $z = -0.4025$; $\sigma_z = 0.1297$; *normal var.* $= -3.1033$.

Table X shows the values of averages of samples of three drawn from the subgroups packed by the first of the two companies, that is, at one of the three canneries from which the samples were derived, while Table XI shows the corresponding data derived from samples packed by the two canneries of the second company. From the first of these tables we find that the value of $\Sigma(y - mx)^2$ is 0.05331, and from the second, 0.08221. From Pearson's formula the corresponding values of $S_{YL}$ are therefore 0.04922 and 0.06257, respectively. These, it will be observed, are slightly more stringent values than those that would have been obtained if the numbers of degrees of freedom had been employed in the calculations.

Application of the sampling limits $\pm 2S_{YL}$ to the line of relation, as illustrated graphically in Figs. 2 and 3, leaves no doubt as to the essential truth of the

TABLE X

CORRESPONDING VALUES OF AVERAGES OF SAMPLES OF THREE DRAWN FROM THE SUBGROUPS OF SAMPLES PACKED BY COMPANY *A*

| Cannery | Variate | Average of three | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| I | pH | 6.33 | 6.22 | 6.21 | 6.28 | 6.30 | 6.31 | 6.39 | 6.33 |
| | O. R.* | 4.00 | 2.00 | 2.00 | 3.00 | 3.33 | 3.00 | 2.67 | 2.67 |
| I | pH | 6.26 | 6.35 | 6.34 | 6.27 | 6.36 | 6.27 | 6.28 | 6.31 |
| | O. R.* | 2.67 | 3.00 | 2.67 | 2.33 | 2.67 | 2.67 | 2.67 | 2.00 |
| I | pH | 6.32 | 6.34 | 6.33 | 6.42 | 6.27 | 6.45 | | |
| | O. R.* | 2.00 | 3.00 | 2.33 | 3.00 | 3.00 | 4.00 | | |

* *O.R. = Odour rating.*

TABLE XI

CORRESPONDING VALUES OF AVERAGES OF SAMPLES OF THREE DRAWN FROM THE SUBGROUPS OF SAMPLES PACKED BY COMPANY *B*

| Cannery | Variate | Average of three | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| II | pH | 6.31 | 6.28 | 6.31 | 6.32 | 6.30 | 6.33 | 6.31 | |
| | O. R.* | 2.33 | 2.00 | 2.00 | 2.33 | 2.00 | 3.33 | 3.33 | |
| II | pH | 6.28 | 6.29 | 6.36 | 6.28 | 6.25 | 6.35 | | |
| | O. R.* | 3.67 | 3.67 | 4.00 | 3.33 | 3.67 | 3.33 | | |
| III | pH | 6.34 | 6.38 | 6.45 | 6.41 | 6.55 | 6.39 | 6.37 | 6.44 |
| | O. R.* | 2.67 | 3.00 | 3.00 | 4.67 | 5.00 | 4.67 | 3.33 | 3.67 |

* *O.R. = Odour rating.*

hypothesis tested, for only one point (Fig. 3) is found outside these limits, and this only slightly beyond the limit. If, as seems reasonable to believe, the distribution of averages of three is approximately normal, we should expect to find, once in 20 times in the long run, a point slightly outside these limits, and this is precisely what is shown in Fig. 3.

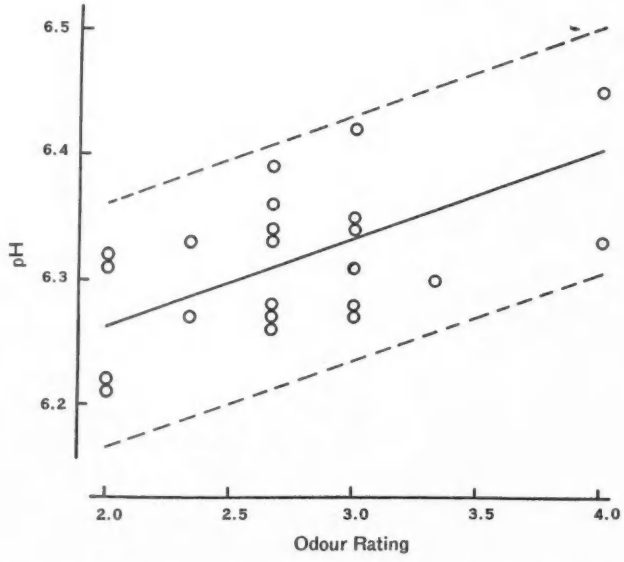


FIG. 2. *Graphical illustration of test of linearity of relation between pH and odour rating by means of sampling limits corresponding to* $\pm 2S_{YL}$ *set on averages of three. Company A.*

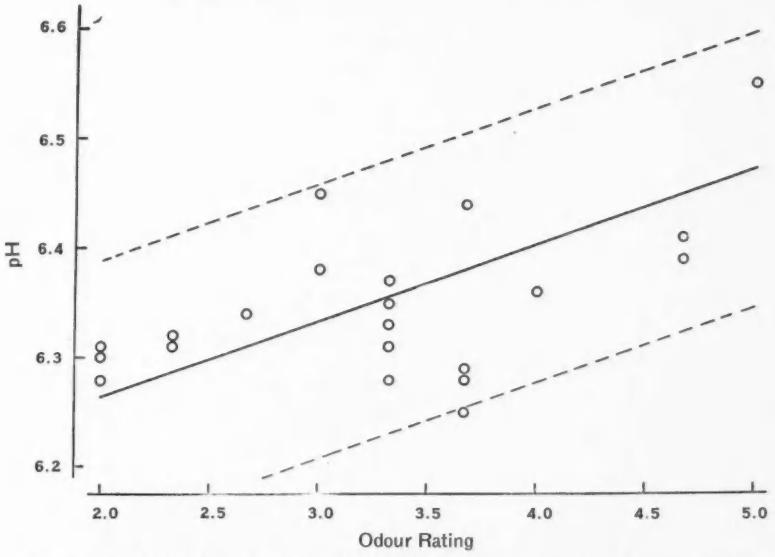


FIG. 3. *Graphical illustration of test of linearity of relation between pH and odour rating by means of sampling limits corresponding to* $\pm 2S_{YL}$ *set on averages of three. Company B.*

## Conclusion

The foregoing results thus indicate that the pH value of fish muscle tissue, or aqueous liquors derived therefrom, is closely associated with three fundamental criteria of freshness, namely, with chemical, bacteriological, and organoleptic criteria. Over the range 6.15 to 6.65 the relation between average pH value and average odour rating in large samples is apparently linear. If, therefore, this relation is confirmed by samples packed in canneries located in other districts, it would afford a simple objective means of grading canned chum salmon with respect to freshness, since, by adjusting the size of the sample taken for examination, the examiner's rating for freshness could evidently be determined from the pH values of the individual samples to any desired level of significance. This follows immediately from the fact that, if $\bar{y}_n$ is the average pH of a sample of $n$, then the standard deviation of $\bar{y}_n$ is $\sigma_n = \frac{\sigma_e}{\sqrt{n}}$. Consequently, if the level of significance is $P$ corresponding to $t\sigma$, the probability is $1 - P$ that the quality of the parcel lies within the limits $\bar{y}_n \pm t\sigma_n$, and hence, that the examiner's rating for the parcel lies within the limits $\frac{\bar{y}_n - \bar{y}}{m} + \bar{x} \pm \frac{t\sigma_n}{m}$, where $\bar{y}$ and $\bar{x}$ are the respective means of the composite distribution.

Before such a method can be safely utilized for practical grading purposes, however, it will be necessary to carry out further work to decide such questions as whether or not the above relation is applicable to samples packed in

other districts, the suitability of this particular examiner's ratings, the ranges in pH and odour rating for other species, and the contribution of the biological condition of the salmon at the time of packing to the examiner's ratings.

## Acknowledgment

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# MARINE MACROPLANKTON FROM THE CANADIAN EASTERN ARCTIC

## I. AMPHIPODA AND SCHIZOPODA[1]

By M. J. Dunbar[2]


## Abstract

Twenty-four amphipod species (one a new species), three euphausiids, and two mysids are recorded from the coastal water of the Canadian eastern Arctic. Most of the records are new.

The list is representative of a high arctic plankton, giving no evidence of the intrusion of Atlantic water. This is in agreement with the hydrographic observations made, and with available hydrographic data from other sources.

The plankton is contrasted with that found in 1936 in Disko Bay, west Greenland, where there appears to be an upwelling of mixed Arctic and Atlantic water. The difference between the plankton of the two sides of Baffin Bay suggests the possibility of distinguishing water of Lancaster Sound (Canadian polar water) from that of west Greenland by means of their planktonic fauna.


## Introduction

There is a great scarcity of plankton material collected from the Canadian Arctic. The expeditions that have worked in this region, notably the Second Norwegian Arctic Expedition of 1898 and the Canadian Arctic Expedition of 1913–1918, have been concerned primarily with other work. The Hudson Bay Expedition of 1921 collected some material from Hudson Bay itself. Danish expeditions have collected plankton in Baffin Bay and Davis Strait, but the emphasis has been on the Greenland rather than the Canadian side. The assumption that species found in waters around Greenland, Spitsbergen, and the Siberian coast are circumpolar in distribution has included the American Arctic with the rest and has made its investigation, as far as the marine life is concerned, appear unnecessary. But to ignore one of the two big outlets from the polar basin, namely the Lancaster Sound current, and to derive knowledge of polar water in large part from the east Greenland outlet only, is not justifiable. To understand the movements of polar water, both within the polar basin and outside it, movements vitally important both to the northern fisheries and to the existence of the Eskimos, it is necessary to know the plankton of the American Arctic, and its investigation therefore becomes a matter of some importance. The present study, a survey of the plankton of the coastal water of the Canadian eastern Arctic, has brought to light several points of interest, both biological and oceanographic, which will be emphasized as they arise in this account. The collections were made in the summers of 1939 and 1940 while the author was a member of the Canadian Eastern Arctic Patrols of those years.

[1] *Manuscript received September 5, 1941.*

*Contribution from the Department of Zoology, McGill University, Montreal, Que., with financial aid from the National Research Council, Ottawa, Canada.*

[2] *National Research Council Student.*

## The Area

The collecting stations are shown in the map (Fig. 1). The majority of the work was done at Lake Harbour, Frobisher Bay, and Clyde River. It will be seen that most of the stations are on the Baffin Island coasts.

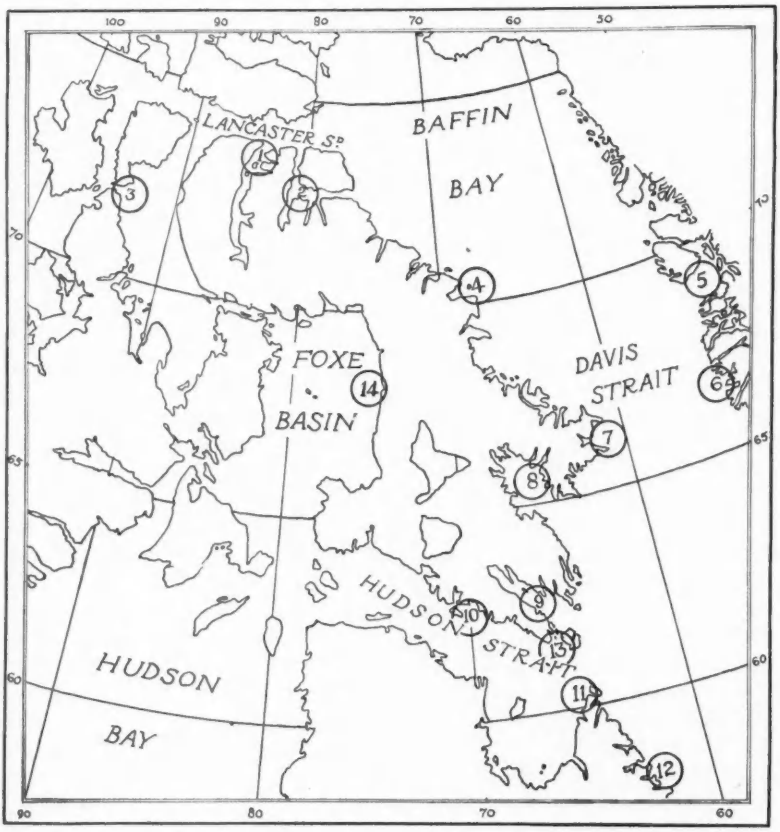



FIG. 1. *Map of the area investigated. 1. Arctic Bay; 2. Pond Inlet; 3. Fort Ross; 4. Clyde River; 5. Disko Bay; 6. Holsteinsborg; 7. Cape Walsingham; 8. Pangnirtung (Cumberland Gulf); 9. Frobisher Bay; 10. Lake Harbour; 11. Port Burwell; 12. Hebron; 13. Gabriel Strait; 14. Hantzsch River.*

## Methods

A motor boat was used at all stations except at Clyde River where, for the two weeks' work, the only boat available was a sailing whaleboat, which did not prove entirely satisfactory. Two nets were used: a one-metre stramin ring-trawl and a silk net of 29 meshes per cm. During the two seasons the following hauls were made.

Stramin:

63 horizontal hauls of 30 min. each, at depths varying from 40 metres to the surface; two oblique hauls from a depth of 75 metres.

5 horizontal hauls of 40 min. each.

6 horizontal hauls of from 7 to 15 min. each.

20 current hauls, using the tidal current to sift the water through the net; time of hauling usually 30 min.

8 vertical hauls, from various depths, all under 50 metres, to the surface.

Silk:

11 horizontal surface hauls of 15 min. each.

3 horizontal surface hauls of 30 min. each.

2 current hauls, surface.

5 vertical hauls, from depths less than 60 metres.

Thus the ordinary lower limit of operations was at 40 metres. At this depth the plankton was found to be scarce in the coastal water, the bulk occurring higher up.

Immediately on being hauled into the boat the catches were concentrated with a small conical concentrating net, and preserved in 10% formalin in sea water, in 4-oz. screw-top bottles. In the laboratory, the preservative was changed to 5% formalin in sea water, and a little borax was added.

## The Hydrography of the Area

The submarine ridge between Cape Walsingham in Baffin Island and Holsteinsborg in west Greenland seems to prevent the passage northward of the bulk of the Atlantic water found in the deeper layers south of the ridge. Reporting on the work of the U.S. Coast Guard expeditions in the *Marion* and the *General Greene*, Smith, Soule, and Mosby (35) calculate that the water in Baffin Bay is derived in approximately equal quantities from the Lancaster Sound current and the west Greenland current. The west Greenland current is shown to be a mixture of east Greenland polar water and Atlantic water, and therefore some Atlantic water must be carried over the Holsteinsborg ridge. North of the ridge, however, it has lost its original physical characters, and its influence in the north and west of Baffin Bay has been assumed to be very small, an assumption that is supported by the results of the *Godthaab* expedition (17, 24) and of the present work. The changes that are occurring in the water off west Greenland will be discussed later; it seems that there is a significant difference between the water of Disko Bay and Baffin Island. For present purposes it is enough to point out that the available hydrographic data seem to indicate that water around Baffin Island is of purely arctic character. From what is known of the arctic indicator species, the collection here described points to the same conclusion.

In 1940, water samples and temperatures were taken at several stations with a reversing-type water bottle and a Richter thermometer. Standard

Copenhagen sea water was obtained from Woods Hole, through the kindness of Dr. Iselin. The results are shown in Table I. The low salinities at Clyde River are due to the presence of the river itself, a circumstance unfortunately unavoidable. The observations were made in order to find out whether there was any trace of non-Arctic water demonstrable hydrographically, within the limits of the scale of the work. It is clear from the table that there is no evidence for the presence of foreign water. These figures agree closely with those of Hachey (8) for the waters of Hudson Strait.

TABLE I

HYDROGRAPHIC DATA, 1940

| Date | Station | Depth, m. | Temperature, °C. | Salinity, ‰ |
|---|---|---|---|---|
| 28/7 | Hudson Strait, 20 miles from land, southeast of Big Island | 75 | −0.81 | 33.62 |
| 28/7 | Lake Harbour anchorage, high tide | 0 | 1.56 | 30.31 |
| | | 10 | 0.33 | 31.07 |
| | | 25 | −0.13 | 31.65 |
| | | 46 | −1.18 | 32.93 |
| 31/7 | Lake Harbour, six miles from anchorage | 0 | 3.68 | 30.74 |
| | | 10 | −0.12 | 31.78 |
| | | 25 | −0.54 | 32.28 |
| | | 50 | −0.66 | 32.58 |
| | | 75 | −0.72 | 32.62 |
| 6/8 | Frobisher Bay | 0 | 1.15 | 30.69 |
| | | 10 | 0.10 | 32.34 |
| | | 25 | −0.04 | 32.34 |
| | | 50 | −0.06 | 32.24 |
| | | 75 | −0.13 | 32.34 |
| 14/8 | Lake Harbour, 10 miles from anchorage | 0 | 2.15 | 31.67 |
| | | 10 | 0.21 | 32.28 |
| 23/8 | Lake Harbour, six miles from anchorage | 0 | 0.85 | 32.50 |
| | | 10 | 0.22 | 32.58 |
| | | 25 | 0.38 | 32.60 |
| | | 50 | 0.35 | 32.72 |
| 11/9 | Clyde River | 50 | −1.39 | — |
| 17/9 | Clyde River | 0 | 0.52 | 27.83 |
| | | 10 | 0.60 | 28.04 |
| | | 25 | 0.55 | 29.88 |
| | | 50 | −1.32 | 32.50 |
| 22/9 | Pangnirtung anchorage | 0 | 1.66 | 29.61 |
| | | 10 | 1.09 | 31.41 |
| | | 25 | 0.75 | 31.71 |
| | | 50 | 0.62 | 32.03 |

## The Plankton

In giving the authorities for the specific names of the animals listed below, the advice and avowed practice of Osgood (21) has been followed in omitting the parentheses around the authorities for forms in which the genus has been changed since the original naming of the species.

Under "records from the American Arctic", records have been included from Alaska to the Newfoundland Labrador, excluding Greenland. Geographically, Greenland is in the American Arctic, but in its history, both political and scientific, it belongs to Europe, and there are good reasons for excluding it from oceanographic arctic America. Most of the species named in this report have been recorded from Greenland waters.

### Order AMPHIPODA

### Suborder HYPERIIDEA

#### Family HYPERIIDAE

**Hyperia galba** Montague. Taken at Lake Harbour and Gabriel Strait. Three specimens.

Records from the American Arctic: Collinson Point, (Alaska (32); Hudson Bay (33) ).

Further distribution: North Atlantic, Spitsbergen, Novaya Zemlya, Murman coast, Greenland, Arctic Ocean; also known from the north Pacific (27, 30).

**Hyperia medusarum** Müller. Taken at Lake Harbour. Five specimens.

Records from the American Arctic: Hudson Bay (32), Cumberland Gulf (36), Labrador (37), and Point Barrow, Alaska (18).

Further distribution: Arctic, north Atlantic southward to about the 55th parallel, and north Pacific (30); California coast (13); commonest in depths between 200 and 1800 metres (30).

**Hyperia spingera** Bovallius. Taken at Lake Harbour and Clyde River. Two specimens. At Lake Harbour, found in the stomach of a ringed seal.

This species is not often collected, and Stephensen (43) has omitted it from the list in Fauna Arctica. Schellenberg (30) gives as its distribution: "Spitsbergen, north coast of Norway, south coast of England, western Ireland, and the Labrador current."

**Hyperia** sp. There were several specimens of this genus that were too small for identification of the species. These were taken at Fort Ross, Arctic Bay, Gabriel Strait, and Clyde River.

**Hyperoche medusarum** Kröyer. Taken at Burwell, Lake Harbour, Arctic Bay, Pangnirtung, Gabriel Strait, and Clyde River. Fifteen specimens. More common than the *Hyperia* species.

In the American Arctic, this species has been recorded from Alaska by the Canadian Arctic Expedition (32), from Labrador (51), and from Newfoundland (6, 23).

Further distribution: West Greenland (9, 44), Gulf of St. Lawrence (34), north Atlantic and adjacent Arctic (30); "possibly circumpolar" (44).

**Themisto libellula** Mandt

Syn. *Euthemisto libellula* Mandt

Taken at all stations, from Hebron to Fort Ross. Several thousand specimens.

In the American Arctic, *T. libellula* has been found at Bernard Harbour and the Dolphin and Union Strait, both free and in the stomachs of *Phoca hispida* Schreber and *Salvelinus malma* Walbaum (32); Hudson Bay (33), and Point Barrow (18).

All workers have commented on the abundance of this form in the Arctic. Tesch (50) says of it that it "shows a marked preference for arctic water". Stephensen (41) records several hundred small specimens from surface water in east Greenland; describing the *Godthaab* expedition material (44), he considers it to be a typically Arctic species. Russell (26) from data supplied by Stephensen, has it in the list of cold water species, but he has omitted it from the list of Arctic indicator species, since it seems able to live, for a time at least, in warmer water. Schellenberg (30) gives its distribution as "in all arctic seas, southward to about 43° N., surface to 2500 metres."

*T. libellula* is without doubt one of the most important organisms in the Arctic, in any habitat, terrestrial or aquatic. It has been shown (5) to be the main food of the ringed seal during the summer at least, and probably for a large part of the year. The full investigation of its biology would be a profitable undertaking. It is comparable, in its position in the economy of the waters, to the key species of the Antarctic, *Euphausia superba* Dana, and seems to take the place, in high latitudes, of the "Krill" (*Thysanoessa inermis* Kröyer and *T. raschii* M. Sars) which form the bulk of the diet of the whalebone whales in the Atlantic.

In the fjord waters, *Themisto* was found to have a vertical optimum noticeably higher in the water than the rest of the zooplankton. It may be that it feeds largely on the phytoplankton, or on the ostracods that were found in large numbers at the surface. At all events, it showed no tendency to sink lower in the water during the daytime, and it was frequently seen and caught in great quantity right at the surface of the sea, even on the brightest of days. It is supposed that this tolerance to bright sunlight is due to its deep purple pigmentation. The pteropod, *Limacina helicina* Phipps, which like *Themisto* is darkly coloured, is also found close to the surface in great profuseness.

**Themisto abyssorum** Boeck

Syn. *Euthemisto abyssorum* Boeck

*Parathemisto oblivia* Kröyer

Taken at Lake Harbour and Hebron. Nine specimens.

American Arctic records: several stations of the Canadian Arctic Expedition on the north Alaskan coast (32); Humboldt Bay, Popof Island, Alaska (14).

Further distribution: Gulf of St. Lawrence (34 and 23), Esquiman Channel (22), and west of Greenland (16); "In all northern seas, in the Atlantic south to about 45° N." (30).

## Suborder GAMMARIDEA

### Family LYSIANASSIDAE

**Anonyx nugax** Phipps. Taken only once, at Gabriel Strait.

Recorded in the American Arctic from Dolphin and Union Strait (32), and from Port Burwell (37).

Widely distributed in arctic and boreal seas, most commonly found in the littoral zone.

**Pseudalibrotus littoralis** Kröyer. Taken at Fort Ross and at Pangnirtung. Eight specimens identified with certainty.

Recorded in the American Arctic from Collinson Point, Alaska, Bernard Harbour, and Dolphin and Union Strait (32), Point Barrow (18), and Hudson Bay (33). Specimens collected by Mr. T. H. Manning off Hantzsch River, west Baffin Island, and sent to the author for identification, belong to this species.

"Circumpolar arctic. 0–1000 m., benthonic; often free-swimming in large swarms." (30).

**Pseudalibrotus glacialis** Sars. Taken at Hebron, Lake Harbour, Fort Ross, Pond Inlet, Arctic Bay, Pangnirtung, Gabriel Strait, Frobisher Bay, and Clyde River. One hundred and eighteen specimens. Also eight specimens from stomachs of ringed seals at Lake Harbour.

American Arctic records: Collinson Point and Point Barrow (32).

Distribution: arctic, pelagic. Recorded from northern Spitsbergen and the Siberian Sea, and from east and west Greenland (30, 28, 44, and 45); also from north Norway (47).

**Pseudalibrotus nanseni** Sars. Taken at Lake Harbour, Gabriel Strait, and Frobisher Bay. Six specimens. Also two specimens from a ringed seal's stomach at Lake Harbour, one a very large female measuring 21 mm.

Recorded from Point Barrow (32).

Further distribution much the same as for *P. glacialis*.

**Pseudalibrotus** sp. Specimens too small and immature to identify with certainty were taken at Lake Harbour and Fort Ross.

The genus *Pseudalibrotus* has given difficulty to taxonomists for some time. The three species listed here, together with *P. birulai* Gurjanowa (7), are all that have so far been reported from the Arctic. *P. glacialis* and *P. nanseni* were described by Sars (28) from material of the Norwegian North Polar Expedition, 1893–1896. In 1917 Stephensen (40) referred to *P. nanseni*, specimens that were immature and these, owing to uncertainty as to the correctness of the determination, he figured in great detail. Later, in working out the material of the Ingolf Expedition, he settled the matter of the determination of the species (41). In identification he relied only on the notch on the inner ramus of the second uropod in *P. littoralis*, and on

the shape of the sixth segment of the second gnathopod in *P. nanseni* and *P. glacialis* (*P. birulai* had not been described). He stated "The characters used in this key seem to me to be the best for determination. They are much better than the coxal plate of pereiopod 1, and the length of the distal part of pereiopod 7 which may vary considerably. Specimens smaller than 4–5 mm. can scarcely be determined."

Sars (28) did not describe the male of *P. glacialis*. Certain differences were recorded in the male by Stephensen (41): "Antennae a little longer. Uropoda almost as in *P. nanseni*, and uropod 3 has natatory setae, not 'without any marginal setae' (Sars). The telson has one pair of apical spines." In the material from Baffin Island, these characters were certainly found in the males, but, with the exception of the long antennae, they were found in many of the females as well. Thus of 12 mature females from Pangnirtung, seven had apical spines on the telson, and eight had one or two long setae on the third uropods, though never as many as in the male. In one male from Pangnirtung, there were no spines on the telson.

Specimens of *P. glacialis*, both male and female, taken in the eastern Arctic, reach somewhat beyond the size limit given by Sars (28). Sars found no specimens of this species above 9 mm. in length. Many of the females in the present collection are longer than this, and there are males measuring as much as 13.5 mm. from rostrum to telson.

With these reservations, the existing descriptions of the species of *Pseudalibrotus* are adequate. Using Stephensen's (41) system, there is no difficulty in distinguishing the species in specimens larger than 5 mm.

Family METOPIDAE

**Metopa longirama** n. sp.* One specimen from Clyde River; a female, with brood lamellae well developed. (Figs. 2 to 11).

General body form close to *M. alderi* Bate; last three pereiopods rather more slender. Antennae long, a little more than half the length of the body. Cephalon roundly produced at the sides, anterolateral corners sharp. Eyes medium, rounded. Coxal plates similar to *M. alderi*. Mouth parts typical of the genus; inner plates of the maxillipeds very small. Finger of first gnathopod long, about two-thirds as long as the sixth joint; fourth joint bearing a row of fine bristles. Second gnathopod, sixth joint ovoid in shape, and large, almost twice as long as the head; two strong spines at proximal end of grasping edge; no emargination. Pereiopods normal, the backward processes on the fourth and fifth joints of pereiopods 4 and 5 (6 and 7 according to Schellenberg's (30) system) not strongly developed. Pleopods very long, the rami two and one-half times as long as the basal portion. Uropods typical of the genus, third uropod with one ramus only. Telson smoothly rounded, with no spinules. Length 4 mm. (female).

Family ACANTHONOTOSOMIDAE

**Acanthonotosoma inflatum** Kröyer. Two specimens, taken at Lake Harbour.

Recorded in the American Arctic from Totness Road, Baffin Island (44), Labrador (37), and Collinson Point, Alaska (32).

Known from the Gulf of St. Lawrence (34), Greenland (42), Spitsbergen (29 and 19). "A true arctic, probably circumpolar littoral species" (44).

Family OEDICEROTIDAE

**Westwoodilla brevicalcar** Goës

Syn. *Halimedon brevicalcar* Goës, in Sars (27)

One specimen taken at Gabriel Strait.

This is the first record of this species from the American Arctic. It was recorded for the first time on the Atlantic coast of Canada by Shoemaker (34), from the collection of the Cheticamp expedition in the Gulf of St. Lawrence.

Distribution: Arctic ocean, Iceland, Greenland (9), and Spitsbergen (29).

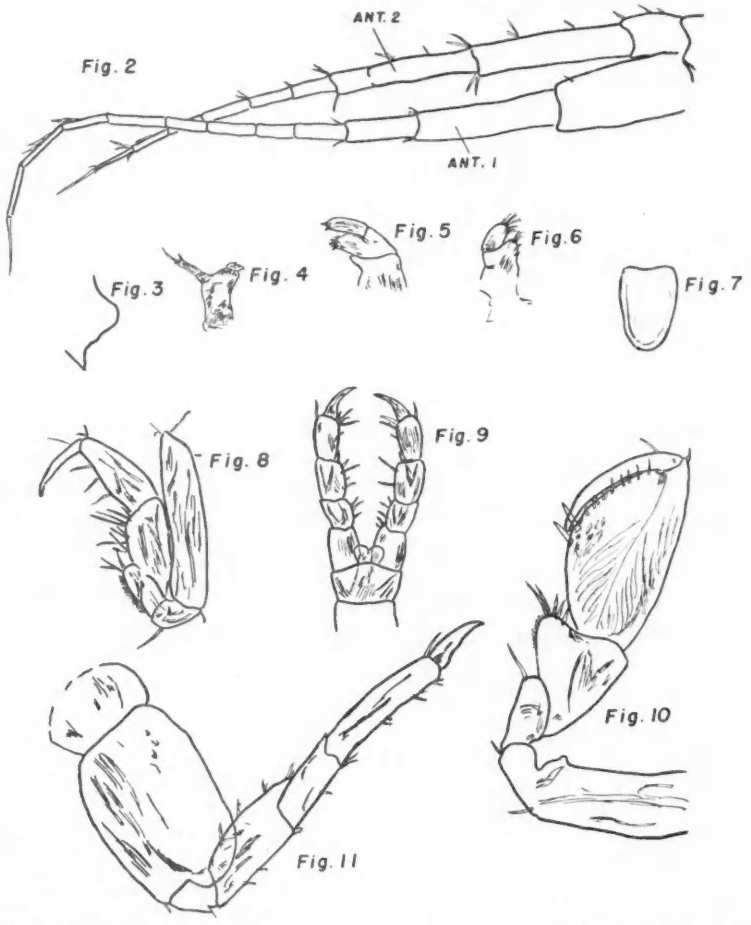
Family CALLIOPIIDAE

**Calliopius laeviusculus** Kröyer. One specimen from Lake Harbour. The specimen agrees with Sars's (27) description and figure, except that the last epimer is somewhat more smoothly rounded, and the legs are not quite so hairy. It is, however, an immature specimen.

American Arctic records: Labrador (37), three stations in Alaskan and western Arctic waters (32), and Hudson Bay (33).

Further distribution: Newfoundland (6), Straits of Belle Isle (22), Gulf of St. Lawrence (34); Greenland, Spitsbergen, Franz Joseph Land, Norway, British Isles, north Pacific (30).

* *The unique specimen and type is in the author's collection, at present in the Department of Zoology, McGill University, Montreal, Que.*




FIGS. 2 TO 11. *Metopa longirama n. sp.* FIG. 2. *Antennae;* FIG. 3. *Cephalon, lateral view;* FIG. 4. *Mandible;* FIG. 5. *First maxilla;* FIG. 6. *Second maxilla;* FIG. 7. *Telson;* FIG. 8. *First gnathopod;* FIG. 9. *Maxillipeds;* FIG. 10. *Second gnathopod;* FIG. 11. *Last thoracic leg.*

**Apherusa megalops** Buchholtz

Syn. *Paramphithoë megalops* Buchholtz (3)
*Halirages megalops* Stebbing (38)
*Apherusa megalops* Shoemaker (32, 33, and 34)
*Halirages megalops* Stephensen (42)

This is not the same species as the *Apherusa megalops* Sars (27) which was first described by Sars as *Halirages megalops*. Shoemaker (34) proposed the name *Apherusa sarsii* for *A. megalops* Sars.

Taken at Hebron, Lake Harbour, Gabriel Strait, and Fort Ross. Twenty-three specimens.

Recorded in the American Arctic from Hudson Bay (33) and Bernard Harbour (32). This latter record, resulting from the Canadian Arctic Expedition, was the first in North America. Up till then the species was not known outside of Greenland waters. Later it was recorded by Shoemaker (34) from the Gulf of St. Lawrence.

**Apherusa glacialis** H. J. Hansen. Taken at Hebron, Lake Harbour, Gabriel Strait, Clyde River, and Fort Ross. Found also in the stomach of a ringed seal at Clyde River. Altogether 42 specimens.

Recorded from Point Barrow, Bernard Harbour, and Harrison Bay (Alaska) (32). A circumpolar Arctic species, known also from the Gulf of St. Lawrence and Newfoundland. Pelagic.

Family PONTOGENEIDAE

**Pontogeneia inermis** Kröyer. One specimen taken at Gabriel Strait.

Recorded from Labrador (37), Hudson Bay (33), Bernard Harbour (32), Gulf of St. Lawrence (34), and Newfoundland (6).

Further distribution: Bay of Fundy, New England coast, Greenland coasts, British Isles, Siberian Sea.

Family PLEUSTIDAE

**Pleustes panoplus** Kröyer. One immature specimen from Lake Harbour. The dorsal carina is not very distinct, and the triangular prominence on the telson is not developed. Otherwise it agrees with the descriptions of Stebbing (38) and Sars (27). Sars mentions that the strong tuberculation of the body is far less distinct in immature specimens than in the adults.

Recorded from Port Burwell (37), and from the Gulf of St. Lawrence (34). Widely distributed in the arctic and subarctic littoral zones.

Family GAMMARIDAE

**Gammarus locusta** Linn. Taken at Lake Harbour, Pangnirtung, Pond Inlet, Arctic Bay, and Fort Ross. Also found in stomachs of ringed seals at Lake Harbour and Clyde River. Twenty specimens.

This species, widely distributed along arctic and northern temperate coasts, has been recorded from Point Barrow (18), Cumberland Gulf (36), Labrador (37), Hudson Bay (33), and from Collinson Point to Dolphin and Union Strait (32).

**Gammaracanthus loricatus** Sabine. Found in the stomach of a ringed seal at Lake Harbour. One specimen.

Recorded from Point Barrow (18), Collinson Point and Bernard Harbour (32), and Hudson Bay (33). Fairly common in arctic seas, but not found further south. It is interesting that no specimen of this species was found by the Cheticamp expedition in the Gulf of St. Lawrence, where so many Arctic forms have been recorded.

Family JASSIDAE

**Ischyrocerus megacheir** Boeck. One specimen from Lake Harbour.

Recorded from Hudson Bay (33) and the Gulf of St. Lawrence (34).

Further distribution: Greenland, Iceland, Spitsbergen, White Sea, Skagerrak, Bering Sea.

**Ischyrocerus anguipes** Kröyer. Nine specimens from Fort Ross, all ovigerous females.

Recorded from Bernard Harbour (32). Widely distributed in the arctic and adjacent seas (44).

Family PODOCERIDAE

**Dulichia tuberculata** Boeck

Syn. *Dulichia curticauda* Sars (27)

One specimen, a male, from Hebron.

The second gnathopods are not quite so large as in Sars's (27) figure, and the basal tooth is not so finely tapered.

An Arctic littoral species (27).

**Dulichia** sp. One female from Fort Ross; a damaged specimen, which cannot be determined with certainty.

## Suborder CAPRELLIDEA

### Family CAPRELLIDAE

**Caprella septentrionalis** Kröyer. Five specimens from Hebron.

Recorded from Cumberland Gulf (36), Labrador (37), and Hudson Bay (33).

Widely distributed: known "from the waters west of Greenland and the New England States to Novaya Zemlya and Denmark; Japan" (44).

## Order EUPHAUSIACEA

### Family EUPHAUSIIDAE

**Thysanoessa inermis** Kröyer

Syn. *Rhoda inermis* Kröyer

One specimen only, from the stomach of a ringed seal at Clyde River.

Recorded in the waters of the American Arctic from Point Barrow, by the Canadian Arctic Expedition (31); also found by the same expedition in the stomach of *Phoca hispida*. Stephensen (46) describes this species as "widely distributed, . . . . . but most frequent in arctic waters". According to Robertson (25) it is the most important food of the cod off Bear Island. It is the most common euphausiid in the Arctic, but was never taken in the nets during the present work. It must be restricted, in the Baffin Island region, to the open water, if it is common at all in that region; it does not occur in the fjords to any great extent. More work is required on the Canadian distribution of this and the following species. On the present evidence, it is impossible to agree with Stephensen that *Thysanoessa inermis* is most frequent in Arctic waters.

This species has been shown to be the staple food of various whales (1). It is found over the whole of the north Atlantic, and has been recorded from the north Pacific (11). In eastern Canada, it has been recorded from several stations in the Gulf of St. Lawrence, Straits of Belle Isle, and Newfoundland waters (49).

**Thysanoessa raschii** M. Sars

Syn. *Rhoda raschii* M. Sars

Taken once in the net, at night, in the open water at the mouth of Frobisher Bay. Also found in seal stomachs at Lake Harbour. Altogether three specimens.

The distribution of this species is much the same as for *T. inermis*. In Arctic America, it has been found at Collinson Point, and from stomachs of *Phoca hispida* and *Salvelinus malma* at Bernard Harbour (31).

**Meganyctiphanes norvegica** M. Sars. Taken only once, in the stomach of a specimen of *Phoca hispida* at Lake Harbour.

No record has been found of this species from Arctic America. Stephensen (46) says of it: "A north Atlantic species, found from east America and Greenland to Siberia and the Mediterranean. It is rarely found in arctic waters." He records it from west Greenland up to 63° 19′ N. and from the coast of King Christian X's Land in east Greenland (45). It has also been found in Disko Bay, approximately 69° latitude, in west Greenland by Hartley and Dunbar (unpublished data). It is known from the Gulf of St. Lawrence, Nova Scotia waters, and Massachusetts Bay (10).

## Order MYSIDACEA

### Family MYSIDAE

**Mysis oculata** Fabricius. Taken at Fort Ross, Hebron, Gabriel Strait, and Pangnirtung; often found in stomachs of *Phoca hispida* at Lake Harbour, Clyde River, and Frobisher Bay (5). Seventy specimens taken in the net, many more from seals.

An Arctic, circumpolar, littoral species. It is nekonic rather than benthonic, being more often taken in nets than in dredges. It has been recorded from all shores washed by Arctic water, including Labrador (37), Bay of Islands, Newfoundland (49), Collinson Point, Bernard Harbour, Dolphin and Union Strait, and Cape Kellett (Banks Island) (31); Spitsbergen (2), Greenland (46, etc.), and also from the west of Canada (48).

**Mysis mixta** Lilljeborg

Syn. *Mictheimysis mixta* Lilljeborg

Found in stomachs of *Phoca hispida* at Lake Harbour and Clyde River, and in the stomach of *P. groenlandica* Erxleben at Frobisher Bay. Nine specimens.

An arctic–boreal littoral species, not hitherto recorded from Arctic America.

## Discussion

Not all the species listed above are truly planktonic. *Acanthonotosoma*, *Gammarus*, *Ischyrocerus*, *Dulichia*, and *Caprella* were all obtained in shallow water, with the net close to the bottom, and are to be regarded as littoral or benthonic forms rather than planktonic.

The list is clearly representative of a high arctic fauna, "high arctic" being used to describe areas in the north where the influence of Atlantic water, or of Pacific water, is negligible or absent. The number of species is small, a character of polar regions, and there is no member that belongs strictly to Atlantic water. *Themisto libellula*, *Pseudalibrotus littoralis*, *P. nanseni*, *P. glacialis*, *Gammaracanthus loricatus*, *Westwoodilla brevicalcar*, *Acanthonotosoma inflatum*, and *Apherusa glacialis* have all been taken as typically arctic species, and, at least if found in the upper layers, as indicative of Arctic water (39). Russell (26) has no amphipod in his list of Arctic indicators, on the grounds that the arctic species are able to survive for some time in water of higher temperature. Stephensen (44) gives four species of amphipods as typical pelagic Arctic forms, namely *Pseudalibrotus nanseni*, *P. glacialis*, *Apherusa glacialis*, and *Themisto libellula*. The remaining species in the present collection are eurythermal forms found in both Arctic and Atlantic areas; individuals from both areas are morphologically identical, species for species, though it has been suggested by Orton (20) that they may be physiologically separate.

A very interesting aspect of the list is the contrast it offers to the analogous fauna in Disko Bay in 1936. Disko Bay (Fig. 1) is in west Greenland, just below the 70th parallel of latitude, and about level with Arctic Harbour and Home Bay, south of Clyde River, in Baffin Island. The distance between the two stations is not great, but the difference in the plankton is remarkable. The place of *Themisto libellula* in the Baffin Island water was taken in Disko Bay by the euphausiids *Thysanoessa inermis* and *T. raschii*. *T. libellula* was not abundant in Disko Bay, and in fact the species *T. abyssorum* was considerably more common than *libellula*. *Meganyctiphanes norvegica* was encountered fairly often, and the mysid *Boreomysis nobilis* G. O. Sars was very common (4).

The "key industry" species in the two regions are *Themisto libellula* in Baffin Island waters and *Thysanoessa* in Disko Bay. Of these, *Themisto* is Arctic in distribution and *Thysanoessa* widely distributed in the Atlantic, if not predominantly so. Of the other Disko Bay forms, *Meganyctiphanes norvegica*, according to Stephensen (46), is a north Atlantic species rarely taken in the Arctic; *Themisto abyssorum*, rare in Canadian Arctic waters and common in Disko Bay, is widely distributed in the Atlantic; *Boreomysis nobilis* is an Arctic species (52), found in deep water, and its presence in Disko Bay and absence in the eastern Arctic coastal water is therefore interesting. Lastly, certain specimens of the chaetognath, *Sagitta elegans* Verrill, which were found to have matured in Disko Bay at a smaller size than the bulk of the population (4), suggest the presence of non-Arctic water.

There is thus evidence for two suppositions; first, that there is an upwelling of mixed Atlantic and Arctic water in Disko Bay, bringing into the bay both Atlantic forms and deep-water Arctic forms, and second, that the influence of Atlantic water at the coast of Baffin Island is very small. The surprising scarcity of *Thysanoessa* and absence of *Boreomysis* in the Baffin Island collections can have two possible explanations; either that these forms do not belong to Lancaster Sound polar water, or that they keep to the deeper water in the Baffin area. Further work on a larger scale than the present will settle this exceedingly interesting point.

This interpretation of the Baffin and Disko Bay amphipod and schizopod plankton is confirmed by the water itself. The water samples taken in Disko Bay were somewhat warmer and contained more salt than the Baffin Island samples shown in Table I. Negative temperatures were met only once, inside the Jakobshavn Icefjord, where negative temperatures would not be surprising. At all other stations the temperatures from 10 to 100 metres ranged from 0.16° to 2.55° C., as against −1.39° to 1.0° C. in Baffin water. Salinities were a little higher in Disko Bay. Thus the salinity at 50 metres varied between 33.00 °/₀₀ and 33.60 °/₀₀ (12), whereas Table I shows salinities of from 32.03 to 32.72 °/₀₀ at 50 metres (excluding the abnormally low value obtained at Clyde River).

Jensen (15) has reviewed the marked warming in climate, both atmospheric and marine, which has occurred in recent decades in west Greenland and eastward. Sea temperatures, up to and including 1936, have been getting higher, and various animals of boreal habit have been extending their ranges to the north, together with a corresponding tendency on the part of the arctic forms to retreat still further north, a circumstance that has made possible, in west Greenland, the establishment of a commercial cod fishery of considerable importance. The plankton found in Disko Bay in 1936 is thus in agreement with this general invasion from the south. In the faunal comparison made above, the plankton of Disko Bay in 1936 is compared with that of the Baffin Island coasts in 1939 and 1940. To the best of the author's belief, there has not been published, in the last five years, any account of a change in the plankton of Disko Bay but, in a postscript to his paper, Jensen gives data that indicate such a change is not impossible. It seems that from 1937 onwards to the date of the paper (1939) temperatures have been falling again, and the fjord cod (*Gadus ogac* Richardson) has again become common in southwest Greenland. It is not known whether the water of east Baffin Island has been behaving in the same way, but the presence of the Lancaster Sound current would render the contingency remote. Such possibilities as this, however, are among the many good reasons from the establishment and maintenance of permanent bases in the Canadian Arctic for the routine continuance of the observation of the sea, both hydrographic and biological.

Whether the comparison made here between the plankton of the two sides of Baffin Bay is considered in three dimensions or four, certain conclusions can be drawn as to the possible use of the species as indicators in the Baffin–

Labrador–Newfoundland area. In the upper layers, *Themisto libellula*, *Pseudalibrotus nanseni*, *P. glacialis*, and *Apherusa glacialis* are reliable indicators of Arctic water. *Boreomysis nobilis*, if found in the upper coastal water, may be taken as indicative of upwelling Arctic water, possibly of Greenland origin. *Thysanoessa inermis* and *Thysanoessa raschii*, being found in both Arctic and Atlantic water, cannot be used to distinguish between the two, but their extreme scarcity at the Baffin Island coast demands further investigation; it is possible that they may be useful to distinguish between Lancaster Sound and west Greenland water.

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